Applications and Development of Organotypic Skin Cultures

SILVYA STUCHI MARIA-ENGLER

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

• Failings of animal model
• Alternative methods at the forefront
• Brazilian Achievements in Epidermal Equivalent
• Organotypic culture: skin as model
• Conclusions
Despite positive testing in animal studies...

- “Currently, nine out of ten experimental drugs fail in clinical studies because we cannot accurately predict how they will behave in people based on laboratory and animal studies.” Mike Leavitt, US Health and Human Services Secretary

Root causes and consequences

- Use of preclinical models that are not able to recapitulate the physiological or pathological processes in humans.
- Novel drug candidates fail to proof their efficacy when tested in humans.
- “Lack of predictively of animal models particularly apparent from the field of drug development”
- “Only 8% of drugs entering clinical phase I gain approved by authorities and half of them fail in phase III”

New Technologies Could Transform Existing
How New Technologies Could Transform Existing Approaches?

• We need human-predictive, rapid and economical methods to evaluate whether or not a compound, no matter if chemical, drug or cosmetic ingredient, is safe and efficacy for intended human use.

• GENOMICS to develop and begin carrying out high- and medium-throughput screening assays to test more chemicals in less time and at less cost.
• COMPUTATIONAL TOXICOLOGY developing new software and methods for predictive toxicology
• SYSTEMS BIOLOGY, a powerful approach that uses computational models and laboratory data to describe and understand biologic systems as a whole and how they operate.
• BIOINFORMATICS, which applies computational techniques to vast amounts of data to understand how cells and cell systems work.
• Microfluidic systems in cell culture (organ on a chip)
• Organotypic cell culture
WHAT ORGANOTYPIC CULTURE DOES?

• Recapitulates phenotypical aspects
• Recapitulates microenvironment physiology
• Interaction among cells and ECM enhances cell differentiation
• Retain the original functions
• Reproduces in vitro similar results to in vivo results

Human context

Maria-Engler et al. J Endocrinol. 2004
WHAT ORGANOTYPIC SKIN CULTURE DOES?

• Study of complex *in vivo*-like behavior of skin cells under *in vitro* settings.
• Allows for the study of human skin using research techniques that are otherwise unsafe and unethical in human subjects.
• Can be used to simulate human skin diseases and study their mechanisms.
• Can be used for screening the therapeutic potential of new drug compounds on human tissues.

LIMITATIONS

• It recapitulates only part of normal skin organization and function.
• Complex, systemic responses, such as wound healing, cannot be reliably studied.
• The size of cultured tissues is constrained by inefficient diffusion of nutrients from the culture medium.
• Studying disease states in vitro requires tissues from disease-affected donors.
WHY THIS IS IMPORTANT IN BRAZIL?
IN VITRO
DERMAL EQUIVALENTS/ SKIN RECONSTRUCTIONS

- BRAZIL HAS LIMITATION TO IMPORT COMERCIAL KITS
- BRAZIL AS A HUGE COSMETIC MARKET
- IMPROVEMENT OF INNOVATION
  - 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)
(3R) RECYCLING SKIN

Foreskin

Separation and digestion

fibroblasts
melanocytes
keratinocytes
DERMAL EQUIVALENT: COLLAGEN+ FIBROBLASTS

+ K e M

KERATINOCYTES AND MELANOCYTES DERMAL EQUIVALENT

AIR LIQUID INTERFACE

2 WEEKS LATER

SKIN RECONSTRUCTED

Collagen IV film

KCs

1 DAY IN MEDIUM

12 DAYS IN AIR-LIQUID INTERFACE

EPIDERMAL EQUIVALENTS
• Epidermal Equivalent in *vitro*

  VMR = validated methods references

Skin USP (2014)  EpiSkin L´Oreal  EpiDerm™ Tissue Model MatTek®  SkinEthic L´Oreal  Human epidermis

• Draize Test

Catarino et al, 2015
Pedrosa et al, 2015
2/17 methods of Brazilian CONCEA RN17, 2014

- OECD TG 431 – Dermic corrosion test
- OECD TG 439 – Skin Irritation test

1. MORPHOLGY AND DIFFERENTIATION/
2. BARRIER FUNCTION
3. VIABILITY
Irritation Test - Epidermal Equivalent

Corrosion Test - Epidermal Equivalent

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride 0.9% (c-)</td>
<td>100</td>
</tr>
<tr>
<td>Acetic acid (+)</td>
<td>90</td>
</tr>
<tr>
<td>(2-bromoethyl) Benzene (NC)</td>
<td>85</td>
</tr>
<tr>
<td>Benzyacetone (NC)</td>
<td>80</td>
</tr>
<tr>
<td>Lactic acid (C)</td>
<td>75</td>
</tr>
<tr>
<td>Octanoic acid (C)</td>
<td>70</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS (C-)</td>
<td>50</td>
</tr>
<tr>
<td>SDS 5% (C+)</td>
<td>50</td>
</tr>
<tr>
<td>Trichlorethylene</td>
<td>(*** )</td>
</tr>
<tr>
<td>Potassium hydroxide</td>
<td>(*** )</td>
</tr>
<tr>
<td>Eugenol</td>
<td>(*** )</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>(*** )</td>
</tr>
</tbody>
</table>
FULL-THICKNESS SKIN MODEL
AND MELANOMA INVASION IN FULL THICKNESS

Keratinocytes

Melanocytes

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

Massaro et al, 2015. USP & Moffitt Cancer Center
AGED/DIABETIC SKIN IN VITRO

Pennacchi et al, 2015.
Skin reconstructs: a tool for pathophysiological models

- Photoprotection and photoaging model (UV effect)

Non-treated

Glicated

• PHOTOPROTECTION AND PHOTOAGING MODEL (UV EFFECT)
Basic Red 51, a permitted semi-permanent hair dye, is cytotoxic to human skin cells: studies in monolayer and 3(D) skin model using human keratinocytes (HaCaT)

Thalita B. Zanoni (1), Manoela Tiago(2), Silvia B de Moraes Barros(2), Alt Bast(3), Geja Hageman(3), Danielle Palma de Oliveira(1), Silvya S Maria-Engler(2) Toxicol Lett. 2014 Jun 5;227(2):139-49.
SKIN BIOLOGY GROUP - FCF - USP

Professor Silvia Berlanga de Moraes Barros
Negative Control NaCl 0,9 %

Viab.: 100%

Viab.: 100%

Viab.: 0,99%

Viab.: 2,84%

Viab.: 82%

Viab.: 80%

Viab.: 8%

Viab.: 2%

Viab.:8,3%

Viab.: 2,3%

2-Bromo etil benzene

Benzil acetone

Viab.: 80%

Viab.: 97%

Viab.: 8%

Viab.: 2%

Viab.:8,3%

Viab.: 2,3%

Láctic Acid

Octanóic Acid

Corrosion Test - Epidermal Equivalent

Viability (%) 100

50

0

3 min 1 hour

Treatment

Sodium chloride 0,9% (c-)
Acetic acid (c+)
(2-bromoethyl) Benzylacetone (NC)
Lactic acid (C)
Octanóic acid (C)