Topical Discussion: Membrane Integrity Testing

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Presentation Plan

Introduction
In-vivo – In-vitro Equivalence
Permeation Devices
Test Methods Overview
Tritiated Water Method (TWP)
Electrical Resistance Method (ER)
Transepidermal Water Loss Method (TEWL)
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Introduction
Membrane Integrity Testing

What kind of membranes?
Membranes that mimic in-vivo human skin in percutaneous penetration research.

Used in practice?
Mostly ex-vivo skin (human & animal)
Also cultured skin & synthetic membranes.

Introduction
Membrane Integrity Testing

What is integrity testing all about?
To verify the in-vivo – in-vitro equivalence of the skin barrier.

Barrier?
The barrier that protects the living cells from the external environment.

What external environment?
Air, sun & all sorts of nasty stuff.

Protects against what?
Water loss, ingress of nasty stuff.
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In-vivo – In-vitro Equivalence

Skin on the Outside

It's variable!
In-vivo – In-vitro Equivalence

Skin on the Inside

It’s complicated!

Fortunately, the main barrier is confined to the top layer, the Stratum Corneum (SC).

In-vivo – In-vitro Equivalence

The Stratum Corneum

Typically ~10-20µm thick.
Layers of dead cells (Corneocytes) surrounded by lipids.
Separates the living cells (wet) from the ambient environment (dry).
In-vivo – In-vitro Equivalence

The Simplified Model

Viable Epidermis → Acceptor Fluid.
Stratum Corneum → Barrier Membrane (could be SC, could be artificial).

NB:-
Lower skin layers could also be present in-vitro (microtomed, full thickness skin ...).
In-vitro perforations may start from the acceptor fluid.
In-vitro hair shafts will be without hair.
Artificial membranes may or may not have perforations.

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Typical static Franz Cells & Flow Cells

Permeation Devices

Cultured Skin
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Test Methods Overview
Three Recognised Methods

<table>
<thead>
<tr>
<th>No.</th>
<th>Method</th>
<th>Permeant</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>TWP</td>
<td>HTO, T₂O</td>
<td>Wet-wet</td>
</tr>
<tr>
<td>2.</td>
<td>ER</td>
<td>Ions</td>
<td>Wet-wet</td>
</tr>
<tr>
<td>3.</td>
<td>TEWL</td>
<td>H₂O</td>
<td>Wet-dry</td>
</tr>
</tbody>
</table>

NB:-
Different permeants:- Methods 1 & 3 use ~water, Method 2 uses ions.
Different measurement conditions:- Methods 1 & 2 are wet-wet, Method 3 is wet-dry.
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Tritiated Water Permeability Method (TWP)
Main Features

- Often seen as the gold standard.
- Wet-wet method, i.e., the skin barrier is not in its natural environment.
- Requires an expensive infrastructure for handling radioactive materials.
- The test takes several hours, but many tests can be run in parallel.
- Measures water permeability.
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Electrical Resistance Method (ER)
Main Features

- Wet-wet method, i.e., the skin barrier is not in its natural environment.
- Requires electrolytes on both sides of the membrane.
- Requires electrodes to be incorporated into the diffusion cells.
**Electrical Resistance Method (ER)**

**Electrical Resistivities of Relevant Electrolytes**

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Electrical Resistivity [Ohm-m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionised Water</td>
<td>$1.8 \times 10^5$</td>
</tr>
<tr>
<td>Fat</td>
<td>25</td>
</tr>
<tr>
<td>Intercellular Fluid</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Pure water has a huge electrical resistivity. Electrolytic current flow is therefore dominant in this method. A membrane would modify this current, depending on its properties. This is the essence of the ER method of integrity testing.

**Electrical Resistance Method (ER)**

**The Key Question**

How do the ions get through the barrier membrane?

The process is electrically driven permeation, but is it:

1. Through the intact membrane?
2. Through the perforations?
3. Through both?
Electrical Resistance Method (ER)

How do ions get through the intact Stratum Corneum in-vivo?

IV. ELECTROLYTE CONTENT OF EPIDERMAL TRANSUDATE

After the inhibition of sweating, the sodium and potassium content of transepidermal transudate is extremely low (Table 1, p. 2148, Ch. 70), the mean concentration of sodium loss was 7.2 ± 2.4 mmol l⁻¹ and the mean concentration of potassium ions was 0.7 mmol l⁻¹. The galvanic skin resistance was greater than 10⁶ ohms after sweating had been inhibited. The method of collection was usually by filter paper but in a few cases determinations were made by ion-specific electrodes.

Answer:- Via the perforations.

The ER method seeks out the perforations.


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Overview

Membrane testing by the TEWL method can be performed using:-
- Unmounted membranes
- Franz cells
- Flow cells
- In-situ cultured skin
- Etc

In all cases, it is important to mimic in-vivo skin, ie the membranes must be:-
- Dry on top
- Wet underneath
- Well acclimatised

Transepidermal Water Loss Method (TEWL)

TEWL is the flux of liquid water diffusing through the SC/barrier. TEWL instruments measure water vapour flux in the adjacent air. You may also get water vapour flux from sweat (in-vivo only) & surface water. Only the TEWL component gives information about the barrier.
Coupling methods

Advantages of the purpose-designed couplings are:
1. Same set-up for testing & permeation.
2. No contact with the membrane.
3. Whole membrane testing.
4. Consistent, calibrated geometry.
5. Reliable sealing to the donor chamber.

Transepidermal Water Loss Method (TEWL)

Coupling to Cultured Skin Samples

Use a contact method to eliminate edge effects.
Use a tube extension to reach down to the sample surface. Control the contact pressure to minimise the risk of damage.

Use a foam rubber support to:
1. Ensure a soft contact.
2. Compensate for minor angular misalignment.
Transepidermal Water Loss Method (TEWL)

Calibration by direct Reference to In-vivo Skin

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Membrane Integrity Testing

TWP:-
Although wet-wet, often seen as the gold standard.

ER:-
Wet-wet. Characterises perforations only.

TEWL:-
Wet-dry.
Can be calibrated in-vitro – in-vivo.
Correlates with TWP.