Surmounting skin barrier: some Insights from Morphology

30+ years of war and still fighting

- ..... And research activity is focused on novel approaches that strive to subvert skin’s excellent barrier function, and broaden the range of active species amenable to percutaneous administration.

Human Skin: Barrier lipids in SC

Slide: Kind courtesy of Dr. Anna Celli, Dermatology Research, UCSF, San Francisco

staining with Nile Red

Skin Barrier and Lipids

- Lipids: the universal waterproofing chemical.
- Specialized lipid enriched organelles: LBs (secreted) Lipid droplets (retained).
- Hydration of SC: opens us channels in mortar lipid domains.
- For those living in water: SC lipids modified (Retention of Glucosylated lipids.); Lipid droplets- Buoyancy as well as metabolic water?
Epidermal triacylglycerol metabolism & maintenance of the skin permeability barrier function


Source of ‘mortar’ lipids

Epidermal lamellar bodies (Containing Cholesterol, Phospholipids, Glycosphingolipids, Enzymes, AMPs) Secreted at SG-SC interface.

## STRATUM CORNEUM LIPIDS: “THE BIG THREE”

<table>
<thead>
<tr>
<th>Species</th>
<th>Approximate wt.%</th>
<th>Molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceramides</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Free Fatty Acids</td>
<td>25</td>
<td>1</td>
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</tbody>
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The “Normal” / Good Barrier
Serving both Barrier & Moisturization

Water ( & a tracer) moves outwards via the Intercellular spaces of nucleated Epidermis.

Meets the lipid barrier here

Water is lost in the vapor form, but is also trapped in corneocytes, plasticizing the keratin.

NMF holds moisture

Lipids ‘seal-in’ moisture, but still allows TEWL

Pro Filagrin

Filagrin

Pro Filagrin

1a: Halkier-Sorensen et al., 1995. BJD.

Altering the SC lipid ratio

- By inhibiting the enzymes for lipid processing: Beta Glucocerebrosidase
- By inhibiting HMG Co A Reductase- the crucial enzyme for Cholesterol synthesis
- Or by Knocking out the enzyme Scd 2 – crucial for TG synthesis
- Can affect the integrity and efficacy of permeability barrier.
Inhibition of Beta Glucosyl Cerebrosidase  
(topical application of Bromoconduritol β epoxide)

Prevents the deglucosylation of probarrier lipids: decrease ceramides

Inhibition of epidermal Cholesterol synthesis  
(Chronic Topical application of statins)

No Change in upper Layers  
Already formed

Occlusion also Interrupts lipid processing

Leads to abnormal LBs and drastic alteration of SC lamellar lipid structure
Fetal impairment of the epidermal barrier in Scd2-/- mice.

Stearoyl-CoA desaturase-2 gene expression is required for lipid synthesis during early skin and liver development.

SCD Knockout mouse skin

Steroyl Co A Desaturase is an enzyme crucial for synthesis of triglycerides.
Relevant to Pharma??

• Statins are the most used drugs today: in many patients, there are side effects-including dry skin.
• An opportunity to counter skin barrier defects with topical HMG CO A activators may exist.

Cholesterol & skin health

• Widely believed: Epidermal Sterologenesis is autonomous.
• Aging: epidermal cholesterol synthesis is reduced, barrier repair is slowed down.
But aging is usually associated with high cholesterol (systemic).

- One of the most talked about and possibly ‘over medicated’ health issue is Cholesterol.
- Regular and prolonged statin treatment at an all time high.
- Prolonged treatment with cholesterol-lowering drugs based on inhibition of HMGCoA reductase does not alter the permeability barrier of the skin (Brazzell, V. et al., Dermatology 1996;192:214–216)

Yet, multiple side effects- including ones on skin, are reported


Statins: Challenges and opportunities ??

• An active topical agent to stimulate epidermal HMG Co A Reductase.
• Exploiting topically applied statins to slow barrier recovery: an adjuvant for effective Transdermal delivery

Control Skin ex vivo
HMG CoA activator compound treated

Potential use in compensating decline in sterologenesis, and strengthen barrier & AMP status

Other side of the coin

• Can we aid transdermal drug delivery via metabolic intervention of epidermal sterologenesis?
• Exploit topically applied statins to slow barrier recovery: an adjuvant for effective transdermal delivery
Fluvastatin as a Micropore Lifetime Enhancer for Sustained Delivery Across Microneedle-Treated Skin

Fluvastatin pretreatment & Microneedle, 7 days later

Hairless Guinea pig skin


Particle mediated gene delivery

With a gene gun, at 350-500 psi, most gold particles (DNA coated) were trapped within human SC. With 800 psi, more particles traverse SC and show up in Epidermis & dermis. SEM of block face: Roger Wepf, ETH Zurich.
Particle-mediated gene delivery

Ex-Vivo skin: 24 hours.
Menon.et.al., 2006.

Encapsulations for delivery
Bicellar systems as vehicle for the treatment of impaired skin


A Biomimetic delivery system?

Somewhat resembles LBs

* Cryo-TEM micrograph of Bicosome® structures (Rodriguez et al. 2010)
Active Delivery systems

- High or low frequency sonophoresis.
- Acoustic pressure waves.
- Particle mediated systems (Powerjet, Gene gun)

A tracer, driven across the skin from the surface, with ultrasound, is uniformly distributed within the extracellular space of nucleated epidermis. However, only a patchy distribution is seen in the SC (short arrows).

Menon et al., 1994. Skin Pharmacol
Tracers distribution

Porcine SC: Quantum dots localization

Low Frequency Sonophoresis
Porcine SC: QDs as tracers

Peer review on pore pathway......

• ...the thorny question of a so-called polar or aqueous pathway across the SC remains unresolved (at least for me). In this particular instance, the experimental and intellectual rigour brought to bear on the intercellular route and its characterization has been lacking.

Guy R.H. 2013. Skin - 'That Unfakeable Young Surface'
Skin Pharmacol Physiol. 26:181-189

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