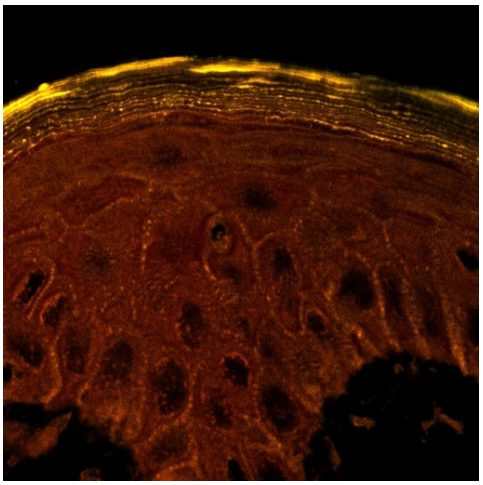
Surmounting skin barrier: some Insights from Morphology

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.

  Wiedersberg,S and Guy,RH. 2014. Transdermal drug delivery: 30+ years of war and still fighting. J. Controlled Release.

Human Skin: Barrier lipids in SC



staining with Nile Red

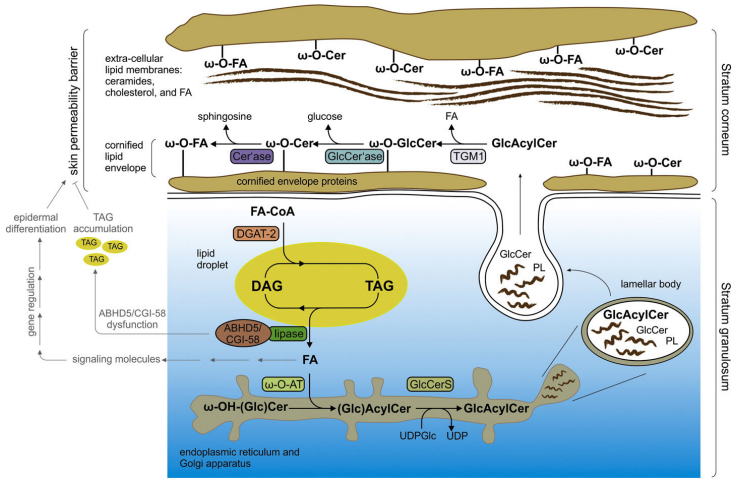
Slide: Kind courtesy of Dr.Anna Celli, Dermatology Research, UCSF, San Francisco

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.);

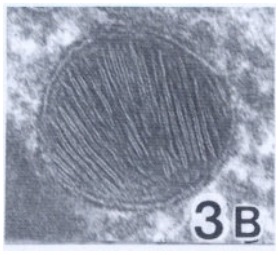
  Lipid droplets- Buoyancy as well as metabolic water ?

Epidermal triacylglycerol metabolism & maintenance of the skin permeability barrier function

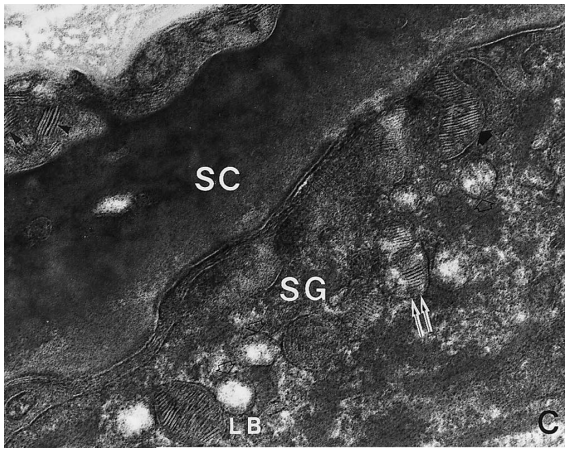


F.P.W. Radner, J. Fischer / Biochimica et Biophysica Acta 1841 (2014) 409–415

Source of 'mortar 'lipids



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

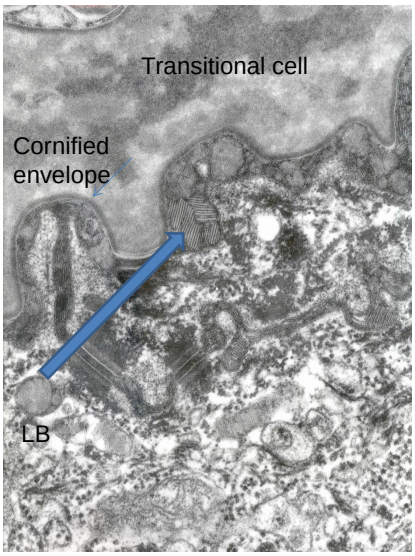


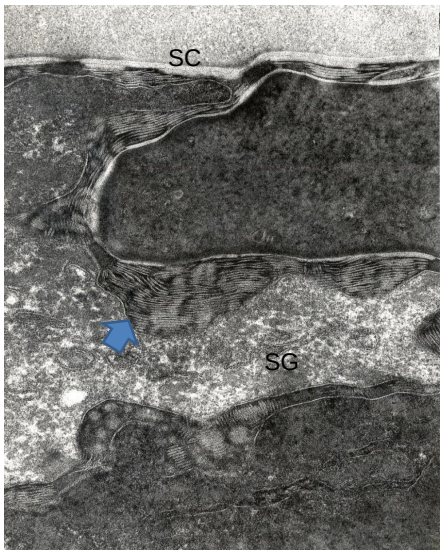
Jiang et.al. Arch Dermatol Res (1998) 290 : 145

STRATUM CORNEUM LIPIDS:
"THE BIG THREE"

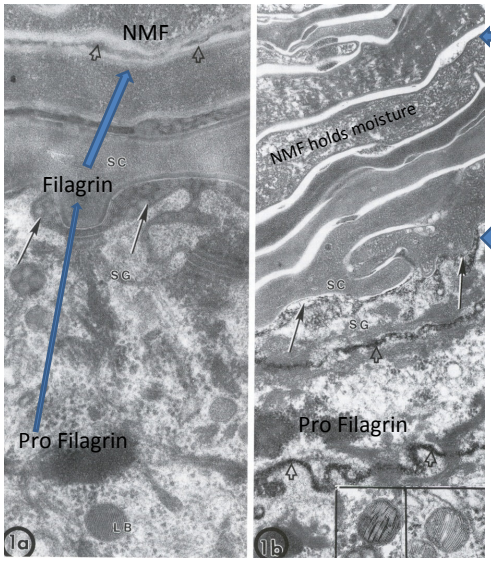
Species	Approximate wt.%	Molar ratio
Ceramides	**50**	**1**
Cholesterol	**25**	**1**
Free Fatty Acids	**25**	**1**

The " Normal"/ Good Barrier





Serving both Barrier & Moisturization



Lipids ' seal-in" moisture, but still allows TEWL

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

Meets the lipid barrier here

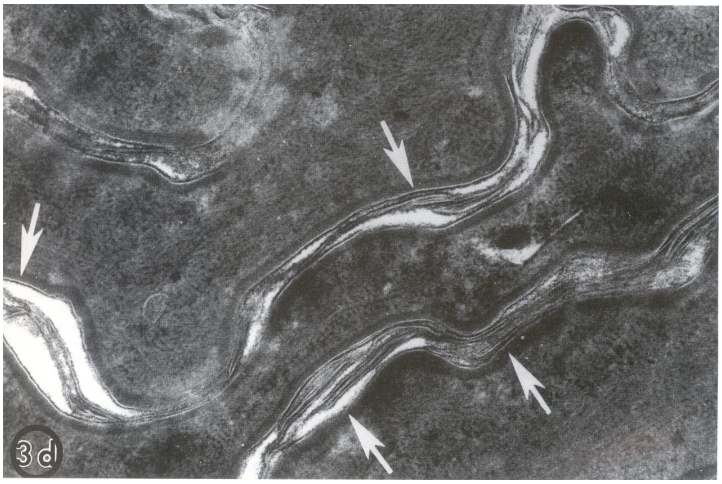
Water (& a tracer) moves outwards via the Intercellular spaces of nucleated Epidermis.

1a: Halkier-Sorensen et.al., 1995.BJD.
1b. Menon & Ghadially, 1997.

Altering the SC lipid ratio

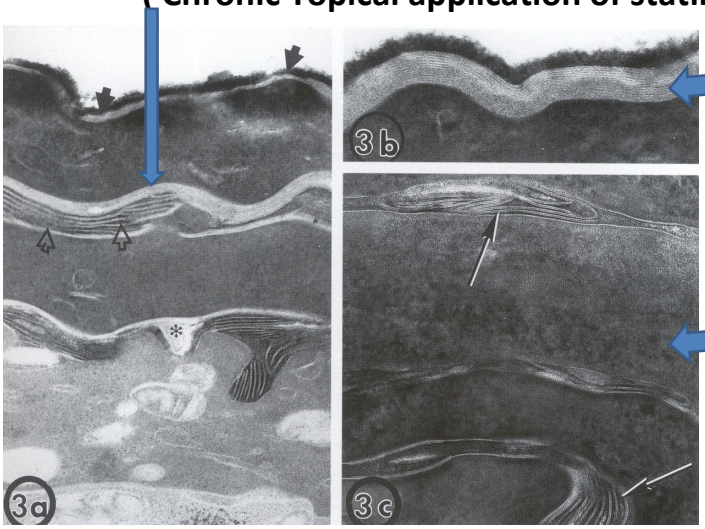
- By inhibiting the enzymes for lipid processing: Beta Glucocerebrosidase
- By inhibiting HMG Co A Reductase- the crucial enzyme for Cholesterol synthesis
- Or by Knocking out the enzyme Scd 2 – crucial for TG synthesis
- Can affect the integrity and efficacy of permeability barrier.

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)

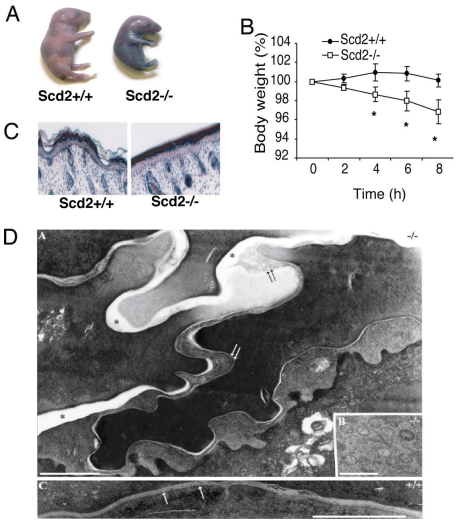


No Change in upper Layers Already formed

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.

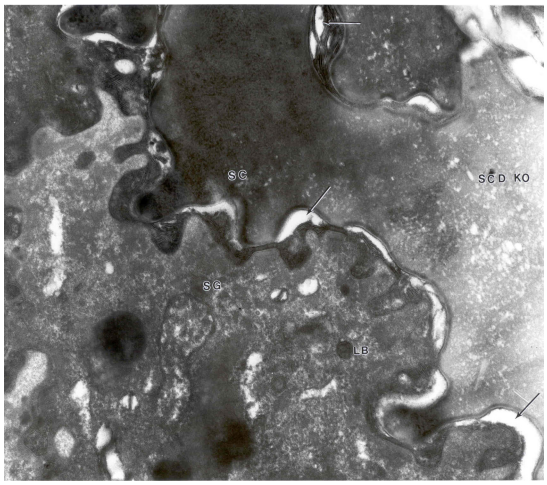


Stearoyl-CoA desaturase-2 gene expression is required for lipid synthesis during early skin and liver development

Miyazaki M et al. PNAS 2005;102:12501-12506

PNAS

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

Krasovec M.· Elsner P.· Burg G. 1993. **Generalized Eczematous Skin Rash Possibly due to HMG-CoA Reductase Inhibitors. Dermatology. 186: 248-252.**

Bliznakov, E. G., and D. J. Wilkins. "Biochemical and clinical consequences of inhibiting coenzyme q (10) biosynthesis by lipid-lowering HMG-Co A reductase inhibitors (statins): a critical overview." *Advances in Therapy* 15.4 (1998): 218-228.

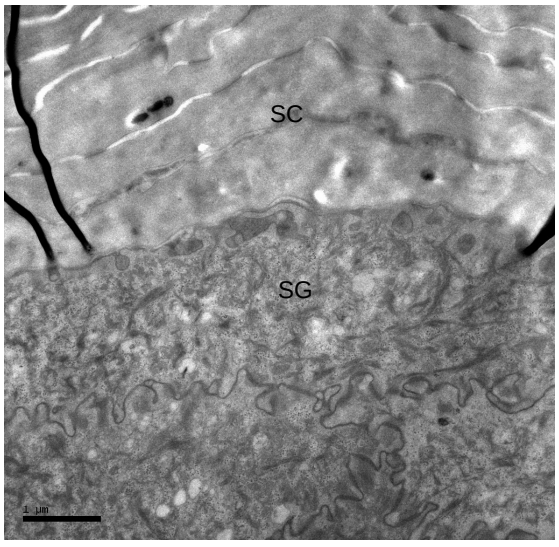
Hydzik,P., Szpak,D. 2011. Side effects of the HMG-CoA reductase inhibitors (statins). Lupus erythematosus induced by Atorvastatin therapy]. 2011. Przegl Lek. 68: 495-498.

Kiortsis,DN. Et al. 2007. Statin-associated adverse effects beyond muscle and liver toxicity. Atherosclerosis. 195: 7-16.

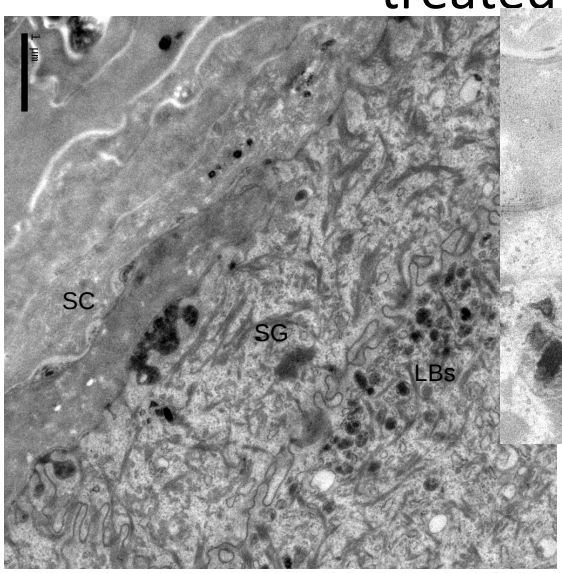
Statins: Challenges and opportunities ??

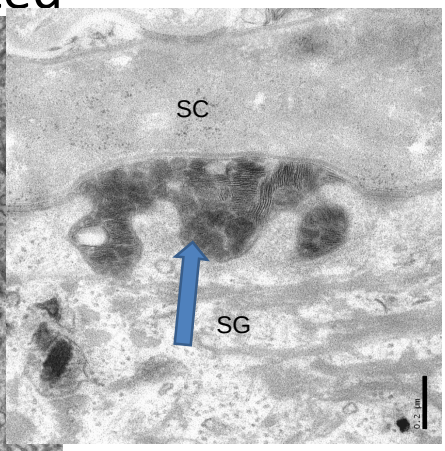
- An active topical agent to stimulate epidermal HMG Co A Reductase.
- Exploiting topically appled statins to slow barrier recovery : an adjuvant for effective Transdermal delivery

Control Skin ex vivo



HMG CoA activator compound treated





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

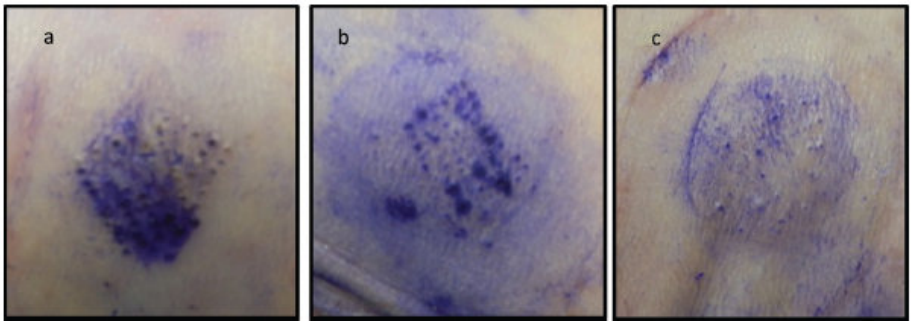
Other side of the coin

- Can we aid transdermal drug delivery via metabolic intervention of epidermal sterologenesis?
- Exploit topically appled 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

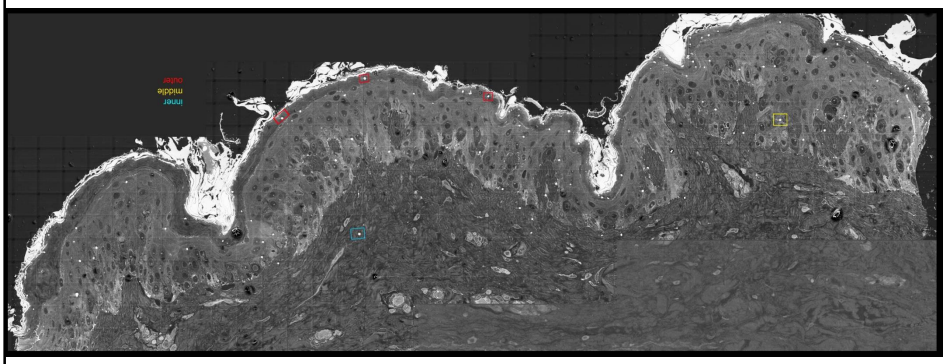
ETOH control



Hairless Guinea pig skin

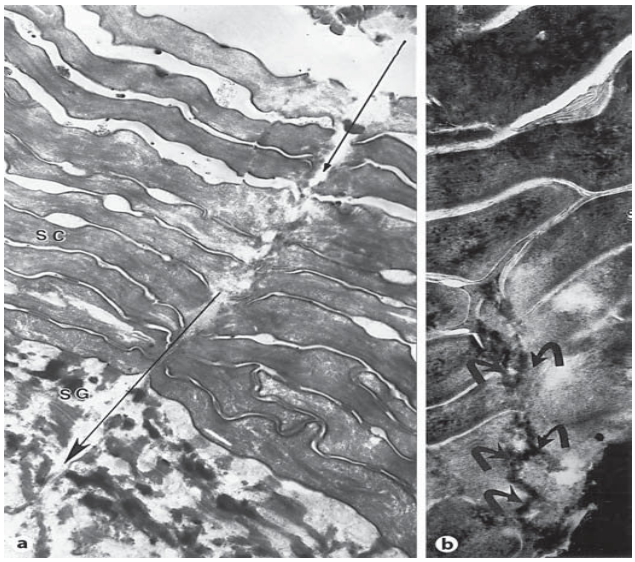
Ghosh, Brogden and Stinchcomb, 2014. J.Pharm. Sci.103: 652-660.

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

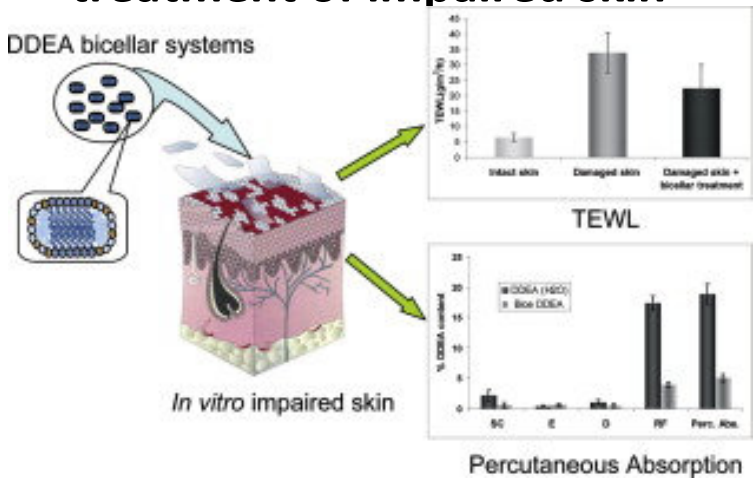


Ex-Vivo skin: 24 hours.

Menon.et.al., 2006.

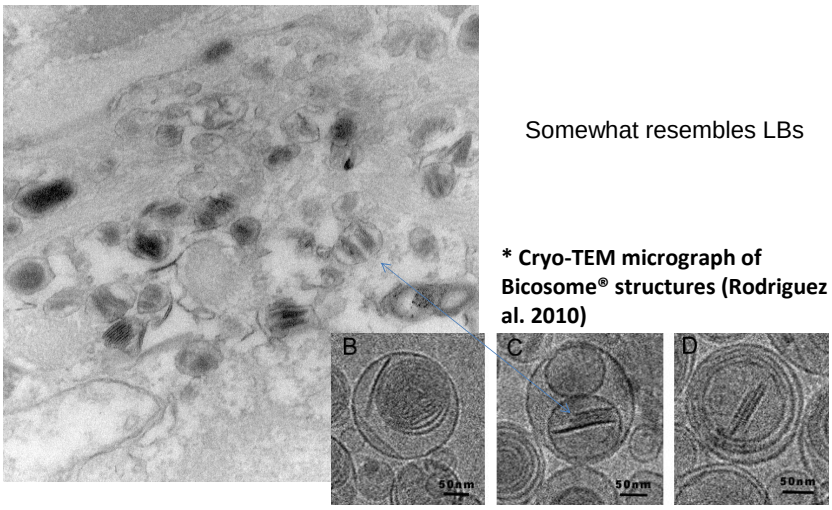
Encapsulations for delivery

Bicellar systems as vehicle for the treatment of impaired skin



Rubio et.al. 2014. European Journal of Pharmaceutics and Biopharmaceutics 86, : 212–218

A Biomimetic delivery system?



Somewhat resembles LBs

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

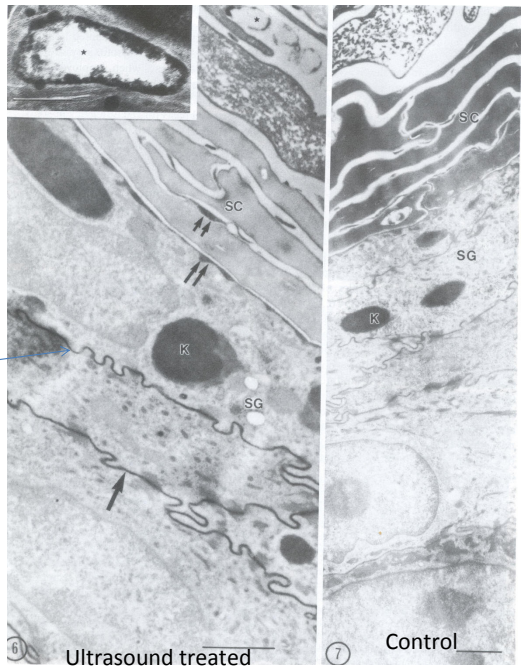
Active Delivery systems

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

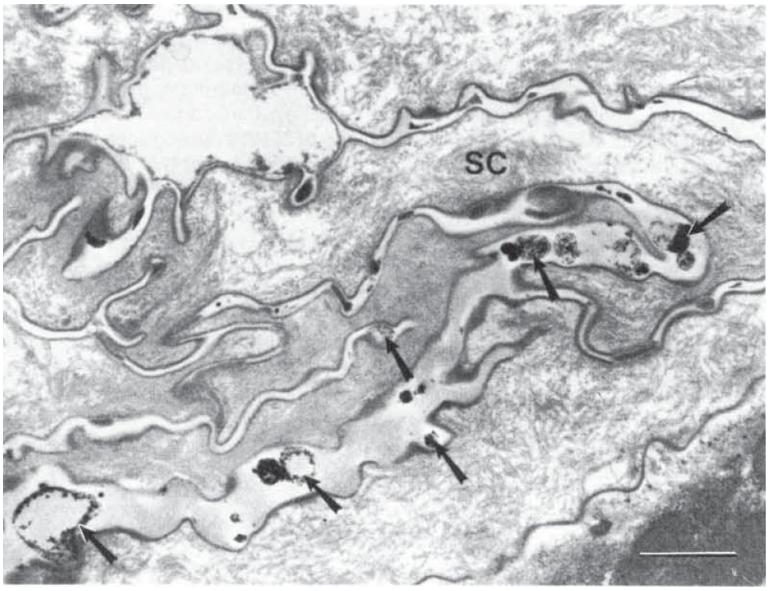
High Frequency Sonophoresis of Tracers

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).

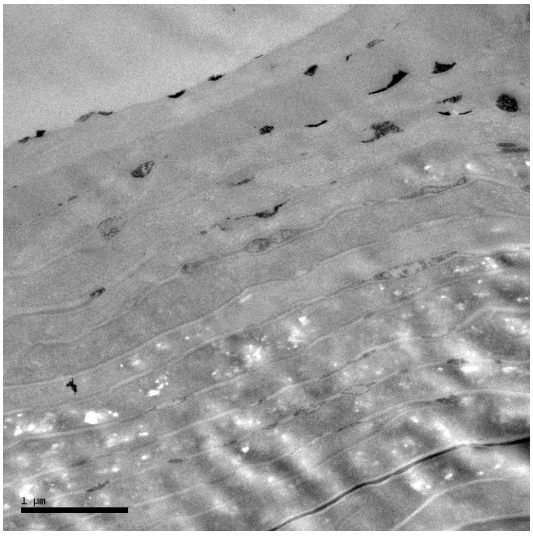
Menon. et al., 1994. Skin Pharmacol



Tracers distribution

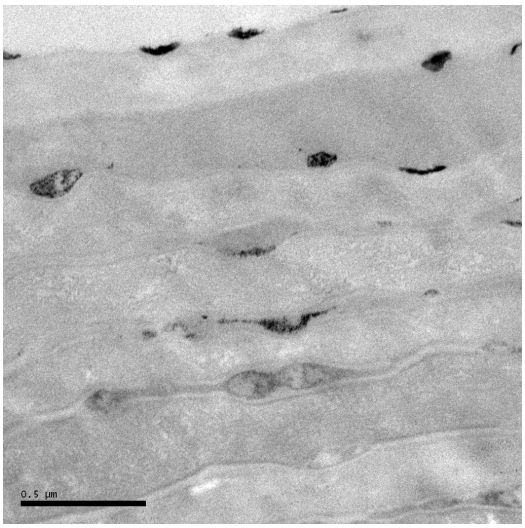


Porcine SC: Quantum dots localization

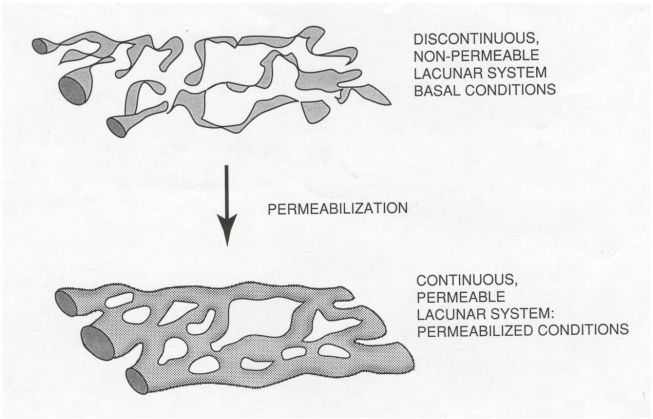


Low Frequency Sonophoresis

Porcine SC : QDs as tracers



Pore Pathway



Menon & Elias, 1997.Skin Pharmacol.10: 235

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

Acknowledgements

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