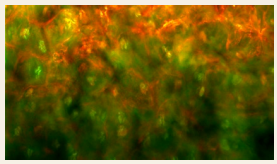
# Predicting, measuring and optimising drug delivery to the skin

**Richard H. Guy**
*University of Bath*

AGAH 6th Dermatological Product Workshop
*London - June, 2015*



Acknowledgements:
Rodrigo Contreras-Rojas, Brian Saar, Sunney Xie, Natalie Belsey, Wing Chiu, Kit Frederiksen, Simon Vanstone, Julian Moger, Natalie Garrett, Gareth Price, Sergey Gordeev, Karsten Petersen, Begoña Delgado-Charro, Annette Bunge, Audra Stinchcomb, Leo Pharma A/S, GSK-Stiefel, U.S. FDA (1U01FD004947-01).

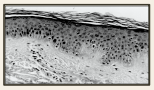
## Introduction

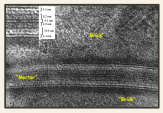
- Drug delivery into and through skin for
  - dermatological therapy,
  - treatment of local, subcutaneous inflammation, or
  - alleviation of systemic disease,

  continues to represent a major challenge.

- While skin barrier function is better understood, and novel technologies are in development…

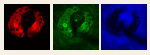
  topical bioavailability remains poor and very difficult to measure.





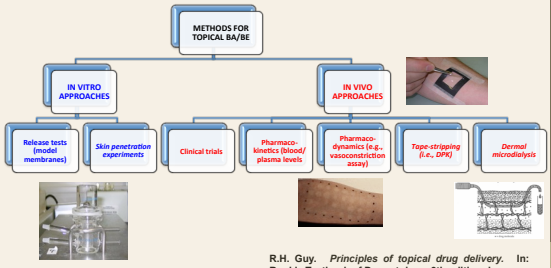
## Key questions

- What are the important rules that must be applied to select the most suitable compound to develop for topical delivery?
  - the potency/permeability conundrum
  - "Lipinski's rules" for skin?
- Determining bioavailability of topically applied drugs for local effect in, or just below the skin?
  - current approaches
  - high-tech Raman imaging



- Are there clear benefits for the application of novel technologies to provide new opportunities for drug delivery to the skin?
  - poration approaches
  - nano-vectors

## Methods for the determination of topical bioavailability/bioequivalence (BA/BE)




R.H. Guy. *Principles of topical drug delivery.* In: Rook's Textbook of Dermatology, 9th edition; in press

## Assessing topical bioavailability: hypotheses…

- Topical BA/BE assessment can be accomplished using appropriately selected *in vitro* and/or *in vivo* surrogate tests.
- All surrogate tests have some, but different, limitations; hence, tests can be complementary.
- Test(s) chosen depend on vehicle complexity and nature of inactive ingredients.

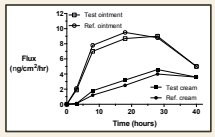
FDA project (U of Maryland, U of Bath, Colorado School of Mines, T. Franz) is designed to ask "what about?"…

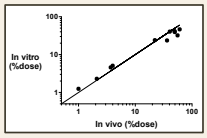
- *In vitro* skin penetration
- DPK (tape-stripping)
- IVRT
- Microdialysis
- Blood levels
- Other approaches (spectroscopic, imaging, etc.)

## In vitro methodologies

1. In vitro release test IVRT
   - artificial membrane (SUPAC-SS)
   - quality control
   - no demonstrated ability to predict in vivo BA/BE

2. In vitro skin penetration experiments
   - long history, substantial data resource
   - yet to gain FDA acceptance, but…




Franz, Lehmann, Raney, *Skin Pharmacol. Physiol.*, 2009, 2011

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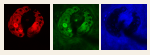

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## Estimation of $J_{max}$

- The absorption of a chemical into the skin depends upon:
  - its physicochemical properties
  - its presentation to the skin (i.e., the 'vehicle' in which it is applied)
  - the 'skin environment', and
  - the duration of exposure.
- Objective:
  - to quantify the maximum absorbability (or flux) of a chemical

$$J_{\max} \cong \frac{D}{\Delta x} * K_{skin/vehicle} * C^{sat}_{vehicle}$$

- D = chemical's diffusivity across skin (typically, stratum corneum)
- Δx = diffusion path-length
- $K_{skin/vehicle}$ = compound's partition coefficient between skin and vehicle,
- $C^{sat}_{vehicle}$ = saturation solubility in vehicle.
- Units of J are amount (e.g., moles or mg) per unit area per unit time.

## Theoretical development

**If the vehicle is aqueous:**

$$J_{\max} \cong \frac{D}{\Delta x} * K_{skin/water} * C_{water}^{sat}$$

**Define a permeability coefficient:**

$$k_p = \frac{D * K_{skin/water}}{\Delta x}$$

**Hence:** $J_{\max} = k_p * C_{water}^{sat}$

**Saturated aqueous solubilities are known, measurable or calculable**

**→ $J_{max}$ can be determined if we can assess $k_p$**

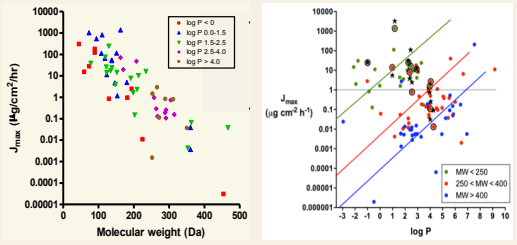
**Algorithm derived by Potts & Guy* from extensive database of ~100 $k_p$ values across human skin in vitro following their application in water:**

$$\log k_p = -2.7 + 0.71 * \log P - 0.0061 * MW$$

**P = octanol-water partition coefficient of chemical; MW = molecular weight**

*R.O. Potts and R.H. Guy. Predicting skin permeability. *Pharm. Res.* 9, 663-669 (1992).

## Prediction of $J_{max}$ (Edetox database, n > 60)



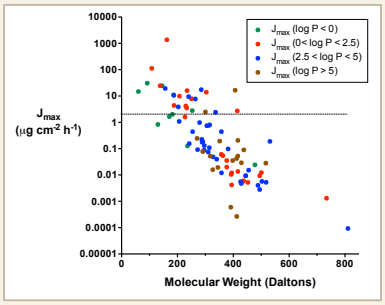

$$\log k_p = -2.7 + 0.71 * \log P - 0.0061 * MW$$

$$J_{max} = k_p * C_{water}^{sat}$$

$$k_p^{corr} = \frac{k_p}{1 + \frac{k_p * \sqrt{MW}}{2.6}}$$

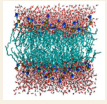
R.H. Guy. *Chem. Res. Toxicol.* 23 (2010) 864-870

## Topical and transdermal drugs (n=92)




## Observations

- **Solubility-diffusion approach provides a decent "first-order" estimate of skin uptake.**
  - **Key parameters are (at least) MW, log P and (aqueous) solubility.**
- **Predictions validated for maximum flux and permeability coefficient; potential also for finite dose, short-contact scenarios.**
  - **Feasibility/success of dermal delivery depends, therefore, on both percutaneous penetration and efficacy of drug.**
  - **A Lipinski-type set of rules is operative.**



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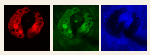

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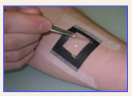
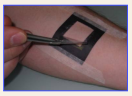
## Dermatopharmacokinetics (DPK) as a test for topical bioequivalence

- **US Food & Drug Administration (FDA)**
  - **Draft Guidance issued June, 1998**
  - **Withdrawn May, 2002**



- **Japanese Division of Drugs**
  - **Issued July, 2003**
  - **Extended November, 2006**



## Topical bioequivalence
## Japanese Division of Drugs

- Guideline for bioequivalence studies of generic products for topical use
- http://www.nihs.go.jp/drug/be-guide%28e%29/Topical_BE-E.pdf
- July 7, 2003
- Dermatopharmacokinetic (DPK) study is acceptable if:
  - **Site of action is either in or below stratum corneum (SC)**
  - **Drug product does not damage SC**
  - **Same concentration of active ingredient (even if in different formulations)**
- Measure at 1 time: steady state after 1 application
- Given that amount of SC stripped by each tape is variable:
  - **Determine amount of SC collected and use average drug concentration (mg/g) instead of drug amount (mg/cm$^2$)**
  - **Or, calculate average concentration from C versus x/L approach**

## DPK of maxacalcitol from ointment and lotion

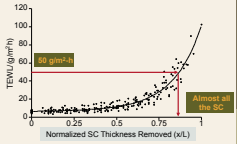
- **Maxacalcitol is 1α,25-dihydroxy-22-oxavitamin $D_3$**
- **Treatment of psoriasis**
- **Compare lotion (generic) to Oxarol ointment (RLD)**
- **Amount of drug is 25 μg/g in both ointment and lotion**
- **Remove SC until TEWL > 50 g/m$^2$-h or 20 tape strips**

**1. Pilot to assess time to reach steady state for lotion and ointment**

| | Lotion (n = 12) | Ointment (n = 12) |
|---|---|---|
| Concentration (μg/g) | 11.2 ± 3.1 | 11.1 ± 3.4 |
| 90% Confidence Interval | 88.9 – 114.6% | |

Umemura K, et al., Int J Clin Pharmacol Ther, 46, 289-294 (2008)

**2. Pivotal assessing bioequivalence at steady state**



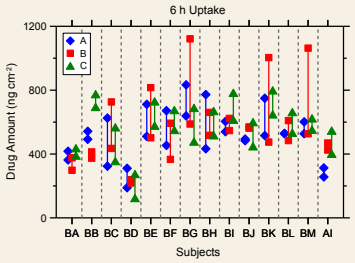

N'Dri-Stempfer *et al.*, Pharm Res, 2009

## "Improved" DPK protocol developed for FDA

- Econazole nitrate cream (1%): 2 generics to reference-listed drug (RLD)
- **4 treatment sites per product (12 sites total)**
  - Duplicate determinations at 2 times
  - 1 uptake time (6 h) & 1 clearance time (17 h); convenient for subjects
- Unabsorbed drug removed using isopropyl alcohol wipes
- **Determined *all* drug in SC by removing most of SC**
  - Removed SC until TEWL was 8-fold greater than pre-stripping value
  - At least 12 tape strips, but not more than 30
  - Tape stripping area < drug application area (control both areas)
- BE of uptake and clearance were assessed separately
- Analyzed tape strips in groups to optimize analytical sensitivity
- Compare within each subject and then across subjects

N'Dri-Stempfer *et al.*, Pharm Res, 2009

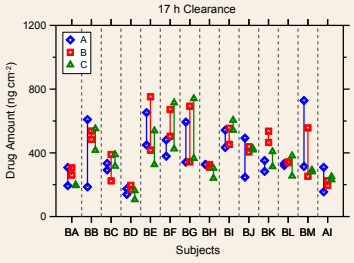
## Econazole uptake into SC




- Drug uptake from 3 clinically BE formulations measured in duplicate (n = 14).
- A = Clay Park. B = Ortho (RLD). C = Taro.
- Duplication of measurements improved results.

N'Dri-Stempfer *et al.*, Pharm Res, 2009

## Econazole clearance from SC

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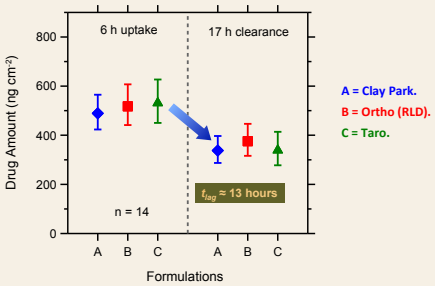
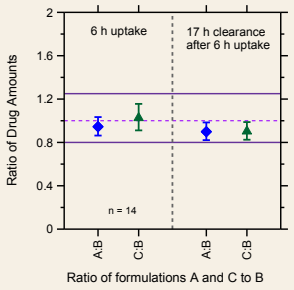
N'Dri-Stempfer *et al.*, Pharm Res, 2009

## Econazole: average drug amounts in SC




N'Dri-Stempfer *et al.*, Pharm Res, 2009

## Econazole: assessment of bioequivalence (BE)




Both A and C were conclusively BE with B after uptake and clearance, evaluated separately.

Only 168 sites (3 products in 14 subjects with replicates for uptake & clearance = 3 x 14 x 2 x 2)

Compare with 1176 sites in tretinoin gel study (3 products in 49 subjects with 8 sites/product = 3 x 49 x 8)

N'Dri-Stempfer *et al.*, Pharm Res, 2009

## "Improved" DPK protocol developed for FDA

- Econazole nitrate creams: 2 (BE) generics to RLD
- 4 treatment
  - Duplica
  - 1 uptake time (6 h) ➔ sufficient drug
  - 1 clearance time (17 h) ➔ sufficient drug and convenient for subjects
- Unabsorbe l tapes included in drug qua
- Determine all drug in SC by removing nearly all of SC
  - Remove SC until TEWL is >8-fold pre-stripping value
  - At least 12 tape strips, but not more than 30
  - Tape stripping area < drug application area (control both areas)
- BE of uptake and clearance determined separately
- Analyze tape strips in groups to optimize analytical sensitivity

Report delivered to FDA in Jan., 2007

WHAT HAPPENED?

No change as yet at FDA with respect to DPK, but new collaborative research in progress

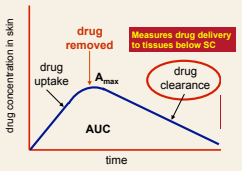
## DPK – current situation?

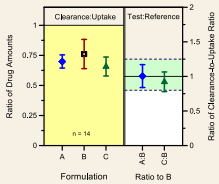
- Improved tape stripping methods can reliably and efficiently assess BE of topical dermatological products
  - Pharmacokinetic (multiple time points) analysis is unnecessary
  - Will FDA ever accept tape stripping to assess BE?
- Remaining questions, clarifications and potential improvements
  - What metric should be assessed?
    - Amount, because adjusting drug quantity by SC mass collected does not reduce variability
    - Determination of SC mass collected and SC thickness not required
    - Eliminates inter-subject variability in SC thickness (compare within subject)
  - Applicability if target tissue is not the SC?
  - What uptake time? How many applications? (Wagner, PQRI-2013)
  - Cleaning excess formulation? Inclusion (or not) of first 2 tape-strips? Yes!

Wiedersberg *et al.*, Eur J Pharm Biopharm, 2009

## DPK – what if stratum corneum is not the target?

- Drug clearance rate is proportional to rate of drug delivery to tissues below SC
- Assessing BE drug delivery to tissues below SC
  - Measure amount of drug after clearance
  - Compare to amount of drug at steady-state before clearance
  - Clearance-to-uptake ratio should be an appropriate metric for assessing delivery to tissues below SC



## Topical BA/BE:

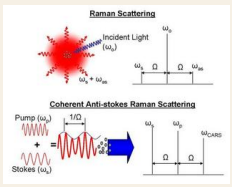
### FDA project (1U01FD004947-01) update and perspectives; application of novel techniques to improve formulations

1. FDA project
   - Betamethasone valerate: DPK on inequivalent formulations ex vivo correlate extremely well with published in vivo (human) data. IVRT does not.
   - Econazole: DPK on uptake of equivalent formulations correlates again with published in vivo (human) data. IVRT of 3 formulations are very similar.
   - Human PK and DPK studies in man comparing different topical diclofenac and acyclovir formulations in progress. To be correlated with in vitro and IVRT.

2. Related research
   - Application of novel technology (Raman scattering microscopy) to better understand formulation behaviour post-application to the skin.
   - Controlling this "metamorphosis" to guide improved formulation conception and development.

## Coherent anti-Stokes Raman Scattering (CARS)

C. V. Raman
(Nobel Prize 1930)




Pump-field is inelastically scattered off molecular vibrations of sample, generating new, red-shifted field components at the Stokes frequencies $\omega_s = \omega_0 - \Omega$

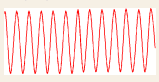
Unlike spontaneous Raman, CARS produces a highly directional field. Two excitation beams ($\omega_p$ and $\omega_s$) form a beating field with frequency $\omega_p - \omega_s$.

When $\omega_p - \omega_s$ matches $\Omega$, all molecules within the interaction volume vibrate in-phase.

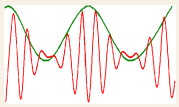
http://newton.ex.ac.uk/research/biomedical/multiphoton/advantages/cars.html

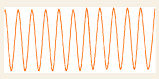
## Coherent Raman Scattering

$\omega_{pump}$

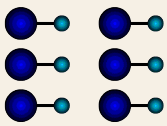


Beating at $\omega_{pump} - \omega_{Stokes}$





$\omega_{Stokes}$



Stimulated excitation of coherent molecular vibration

$\omega_{pump} - \omega_{Stokes} = \omega_{vib}$

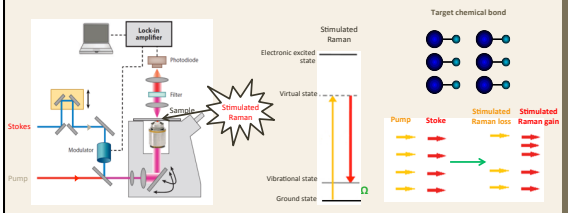
VOLUME 82, NUMBER 20 PHYSICAL REVIEW LETTERS 17 MAY 1999

**Three-Dimensional Vibrational Imaging by Coherent Anti-Stokes Raman Scattering**

Andreas Zumbusch,* Gary R. Holtom, and X. Sunney Xie†
*Pacific Northwest National Laboratory, William R. Wiley Environmental Molecular Sciences Laboratory, P.O. Box 999, K8-88, Richland, Washington 99352*
(Received 9 December 1998)

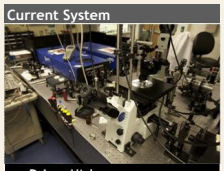
A multiphoton microscopy based on coherent anti-Stokes Raman scattering is accomplished with near-infrared ultrashort laser pulses. We demonstrate vibrational imaging of chemical and biological samples with high sensitivity, high spatial resolution, noninvasiveness, and three-dimensional sectioning capability. [S0031-9007(99)09110-3]

## Stimulated Raman Scattering



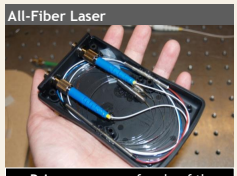

- Images the specific chemical bond of interest
- Signal is linearly proportional to concentration of target molecule
- Information on penetration depth and pathways of multiple components of a formulation

**Current System**



- **Price**: High
- **Robustness**: Requires optical table
- **Weight**: 200kg
- **Cooling**: Water-cooling
- **Power-consumption**: High

**All-Fiber Laser**



- **Price**: economy of scale of the telecom industry
- **Robustness**: no adjustment after splicing
- **Weight**: <2kg
- **Cooling**: Air-cooling
- **Power-consumption**: Battery possible

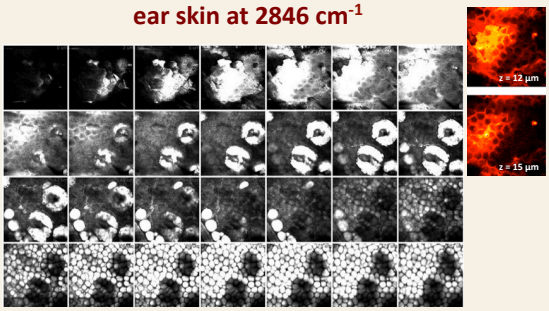
C. Freudiger & Sunney Xie
Invenio Imaging, Inc. & Harvard University

## Advantages of CRS


- Raman resonance enhancement provides chemical selectivity without need for labelling.
- There is little scattering of the near-infrared excitation beams, allowing deep penetration in tissues.
- Due to anti-Stokes shift, CARS signal is of shorter wavelength than one-photon fluorescence.
  - allows detection in presence of a strong fluorescent background.
- Coherent addition of CARS fields generates a large signal.
- Nonlinear dependence on excitation intensities -> inherent 3D resolution.
- Low absorption of near-infrared excitation beams significantly reduces photodamage in biological samples.

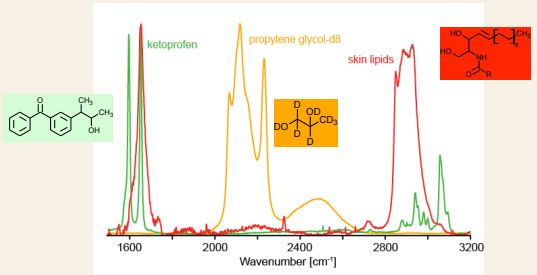
http://newton.ex.ac.uk/research/biomedical/multiphoton/advantages/cars.html

## SRS $CH_2$ images of mouse ear skin at 2846 $cm^{-1}$



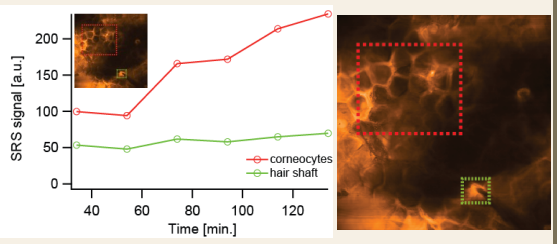

BG Saar, LR Contreras-Rojas, XS Xie, RH Guy, Molecular Pharmaceutics 8, 969-975 (2011)

## Raman spectra of key chemical species




SRS contrast is based on spontaneous Raman spectra, which are used to determine optimal excitation wavelengths: 1599 $cm^{-1}$, 2120 $cm^{-1}$ and 2845 $cm^{-1}$ report on ketoprofen, deuterated PG and skin lipids, respectively.

## SRS analysis of skin penetration pathways

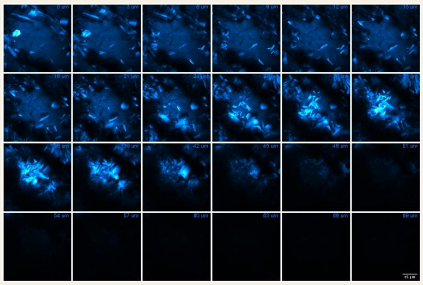



* Temporal profiles of PG-d8 at z = 6 µm from two distinct regions
* Intercellular penetration increases steadily with time
* Follicular transport already 'saturated' at 26 mins post-application
* Scheuplein was right!

BG Saar, LR Contreras-Rojas, XS Xie, RH Guy, Molecular Pharmaceutics 8, 969-975 (2011)

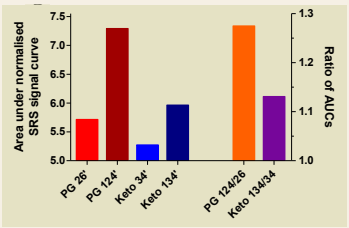
## Metamorphosis of a formulation

Ibuprofen-d3 dissolved in propylene glycol at close to saturation



BG Saar, LR Contreras-Rojas, XS Xie, RH Guy, Molecular Pharmaceutics 8, 969-975 (2011)

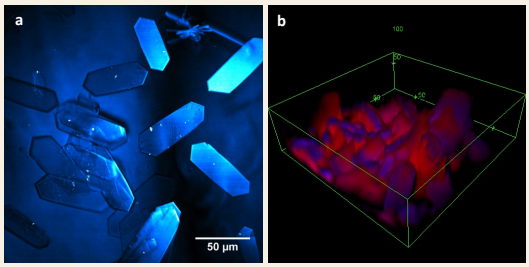
## Depth-profiling analysis of skin penetration




* Comparison of relative penetration of PG-d8 and ketprofen over the course of the experiment (~2 hr) [left-hand axis]
* Ratio of AUCs (measured near the beginning and end of the exposure) indicate faster permeation of PG relative to ketoprofen [right-hand axis]

BG Saar, LR Contreras-Rojas, XS Xie, RH Guy, Molecular Pharmaceutics 8, 969-975 (2011)

## Metamorphosis of a formulation




Crystals of Ibuprofen-d3 formed on skin within 0.5 hr post-application.

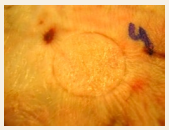
Panel (a): SRS contrast at 2120 $cm^{-1}$ (C-D stretch).

Panel (b): 3D projection of C-D contrast (blue, for ibuprofen) and $CH_2$ signal from skin lipids (red) to reveal topology.

N. Belsey et al., J. Control. Release 174 (2014) 37-42

## Polymeric film-forming systems (FFS)

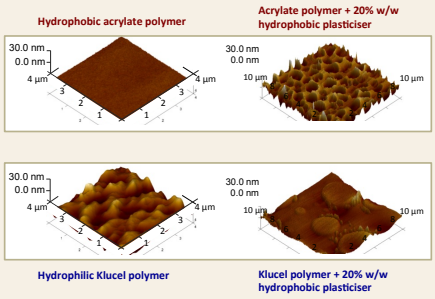
- Film Forming System (FFS): drug, polymer, solvent + plasticizer/lipid.
- A polymeric solution is applied to the skin and forms a thin, transparent and flexible film on the skin.



**Advantages over conventional dosage forms:**

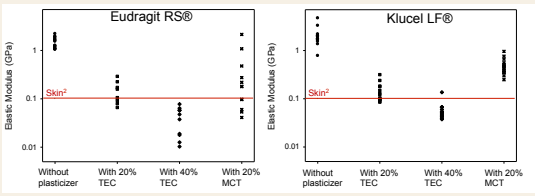
- Cosmetically acceptable
  - invisible and flexible on the skin.
- Application convenience
  - short drying time (<5 min) and rub off resistance
- Less frequent dosing
  - prolonged and/or increased delivery

## Atomic force microscopy (AFM) - imaging




H. Garvie-Cook, K. Frederiksen *et al.* J. Control. Release (2015) in press.

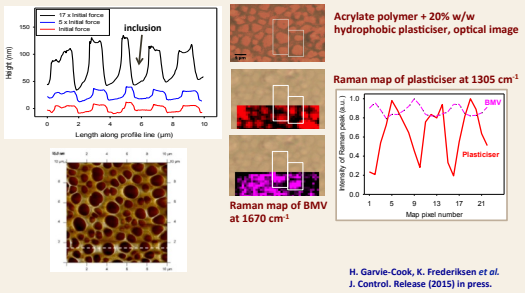
## Nanoindentation to determine elastic modulus




- Elastic modulus decreases with increasing plasticizer content
- Permits elastic modulus of FFS to be matched to that of skin
- Further work undertaken with MCT

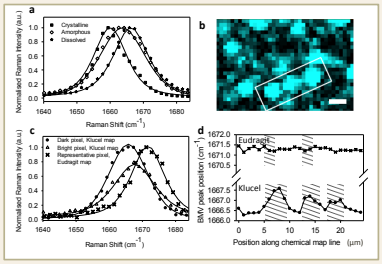
H. Garvie-Cook, K. Frederiksen *et al.* J. Control. Release (2015) in press.

## Polymeric film-forming systems - AFM and Raman mapping




H. Garvie-Cook, K. Frederiksen *et al.* J. Control. Release (2015) in press.

## Physical state of BMV in acrylate and hydrophilic polymers with MCT plasticiser




- BMV appears to be "dissolved" in the acrylate polymer, but in the solid state in the hydrophilic, Klucel FFS
- Skin uptake of BMV is greatest from acrylate FFS with MCT

H. Garvie-Cook, K. Frederiksen *et al.* J. Control. Release (2015) in press.

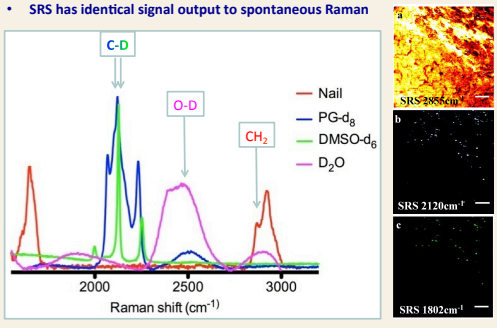
## The challenge of delivering actives to the nail

- Compact structure
- 80 – 90 layers of dead keratinized cells
- S-S bond
- Hard keratins
- Low lipid content 0.1-1%

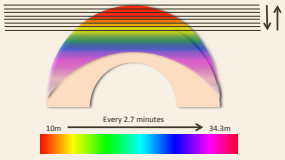
* Fungal infections
* Nail psoriasis

## Solvent permeation into the nail

- Deuterated solvents used to achieve image contrast
- SRS has identical signal output to spontaneous Raman

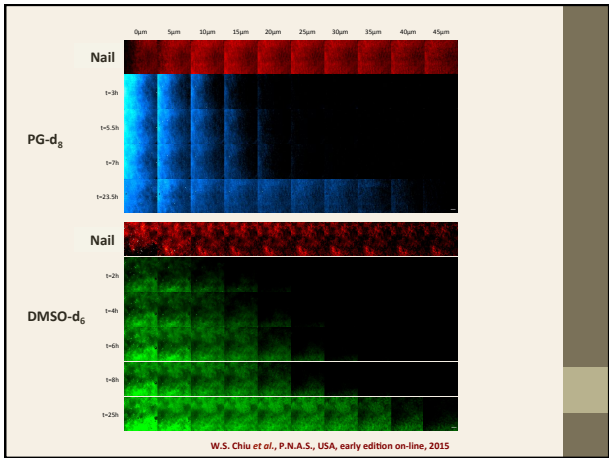



## Absorption of $D_2O$



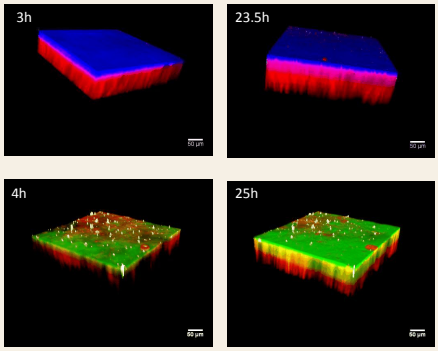

Cross-sectional view


W.S. Chiu *et al.*, P.N.A.S., USA, early edition on-line, 2015



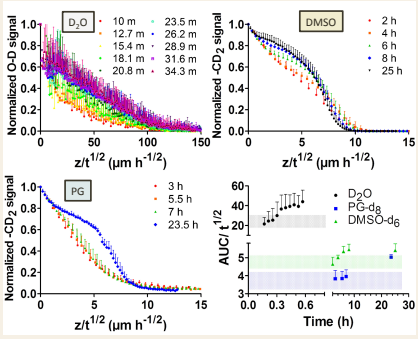

W.S. Chiu *et al.*, P.N.A.S., USA, early edition on-line, 2015

## PG-$d_8$ and DMSO-$d_6$ in nail



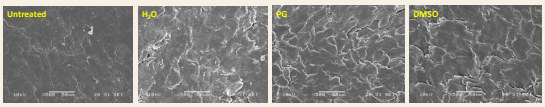

W.S. Chiu *et al.*, P.N.A.S., USA, early edition on-line, 2015

## Concentration/time/position-dependent diffusion




W.S. Chiu, P.N.A.S., USA, in press, 2015

## Effect of solvent uptake on nail integrity

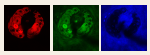



- Solvents clearly undermine integrity of the nail (scale bar = 50 μm)
- Effects are apparently concentration and time-dependent
- Kinetics of water diffusion and its impact on the nail are much more rapid than those of PG and DMSO
- Diffusivity of water estimated to be 25-40 times greater than that of the other two solvents

W.S. Chiu *et al.*, P.N.A.S., USA, early edition on-line, 2015

## Key questions

- What are the important rules that must be applied to select the most suitable compound to develop for topical delivery?
  - the potency/permeability conundrum
  - "Lipinski's rules" for skin?
- Determining bioavailability of topically applied actives for local effect in, or just below the skin?
  - current approaches
  - high-tech Raman imaging

- **Are there clear benefits for the application of novel technologies to provide new opportunities for delivery of actives to the skin?**
  - **poration approaches**
  - **nano-vectors**

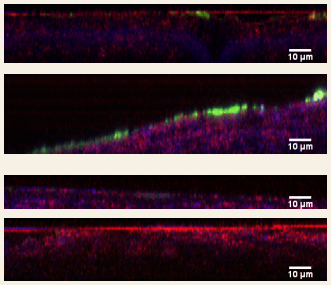


## Enhancing transdermal transport

- **Passive diffusion of molecules > 1000 da is very inefficient**
- **Skin's principal function is to provide a barrier**
- **Efficient transdermal transport ⇒ enhancement technology**
  - which acts on the molecule
    - iontophoresis
  - **which acts on the barrier**
    - **microneedles, microporation**
    - ultrasound
    - other 'permeabilization' approaches (e.g., high-velocity particles)
  - **which involves novel formulation**
    - liposomes, **nanoparticles,** or other carrier/targeting moiety, or enhancers

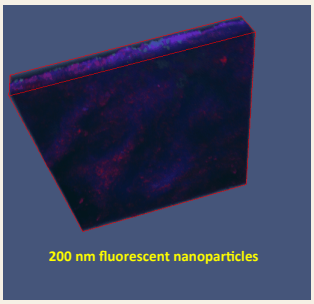

## Nanoparticles do not penetrate intact (or even partially compromised) skin!

20 nm fluorescent particles applied to 4x tape-stripped skin for 16 hours -> no penetration!




C. Campbell, L.R. Contreras-Rojas et al., J. Control. Release, 162: 201-207 (2012).

## Reconstructed 3-D confocal stack

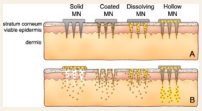


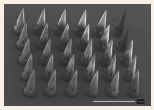

Red/blue: Skin autofluorescence

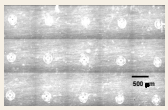
Green: Fluorescent nanoparticles

C. Campbell, L.R. Contreras-Rojas et al., J Controlled Release 162 (2012) 201-207

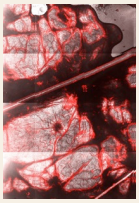
## Microneedle poration of skin

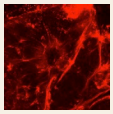


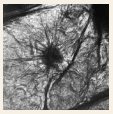



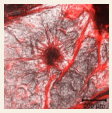



1st example examined: metal µneedle arrays, 350 and 125 µm.





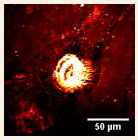




350 µm microneedle array applied to porcine skin.
Exposure to Nile Red for 5 minutes, then imaged at 543 nm by confocal microscopy (monochrome panel is light reflectance). Tile size = 900 x 900 µm$^2$.

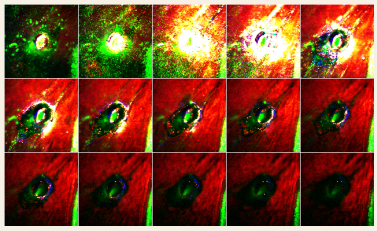
N. Belsey et al., J. Control. Release 174 (2014) 37-42

## Microneedle poration of skin



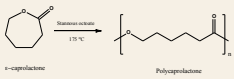

$CH_2$ contrast: nanoparticles remaining in hole created by microneedle.

Microneedles coated with fluorescent particles.
Green = TPF. Blue = SRS retinol
Red = SRS skin lipids

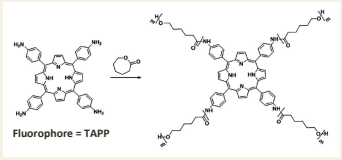


Belsey *et al.* unpublished data

## Fluorescently-labelled polycaprolactone (PCL) nanoparticles containing octyl-methyl cinnamate (OMC)



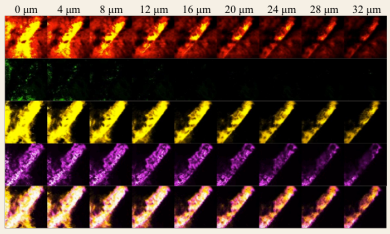

Average nanoparticle diameter ~145 nm
Polydispersity index = 0.24
OMC encapsulation efficiency ~90%
[OMC] = 23 mg/mL




Nanoparticles tracked by 2-photon fluorescence
OMC at 1601 cm$^{-1}$; -$CH_2$ signal from nail at 2901 cm$^{-1}$

W.S. Chiu, PhD thesis

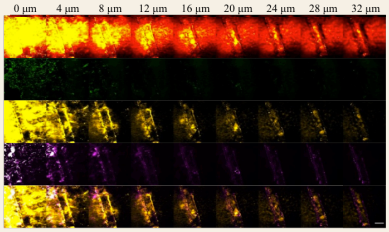
## OMC-loaded nanoparticles on microneedle-treated nail (2 hours)




Nail protein ($CH_2$). Off-resonance (background)
Octyl-methyl-cinnamate (OMC)
2-photon fluorescence from TAPP-labeled nanoparticles
Overlay of OMC and TAPP.

W.S. Chiu, PhD thesis

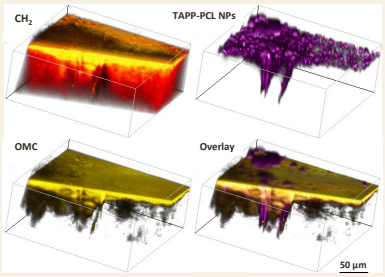
## OMC-loaded nanoparticles on microneedle-treated nail (7 days)




Nail protein ($CH_2$). Off-resonance (background)
Octyl-methyl-cinnamate (OMC)
2-photon fluorescence from TAPP-labeled nanoparticles
Overlay of OMC and TAPP.

W.S. Chiu, PhD thesis

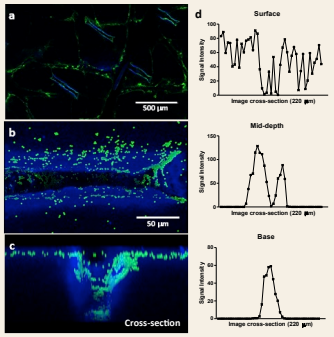
## OMC-loaded nanoparticles on microneedle-treated nail




W.S. Chiu, PhD thesis

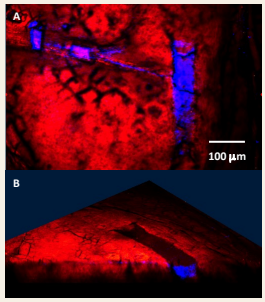
## Fluospheres on porated skin

- Thermal ablation: small pores/channels (~ 300 µm long, 100 µm deep) created by short bursts of heat.
- Poration device utilizes a microarray of metal filaments.
- Skin auto-fluorescence is heightened around pores.
- Particles have an affinity for the pore surfaces.
- Signal intensity profiles at surface, mid-pore and bottom of 'trench'.

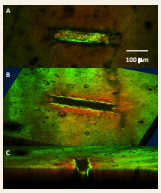



N. Belsey et al., J. Control. Release 174 (2014) 37-42

## Deuterated nanoparticles: Stimulated Raman scattering (SRS)

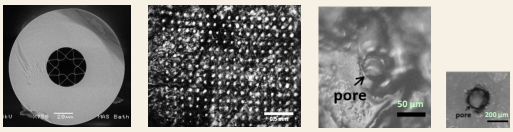



- 40 nm diameter deuterated methyl methacrylate particles
- CARS (red) $CH_2$ stretching contrast at 2855 $cm^{-1}$
- SRS (blue) CD contrast; fluorescein
- Images prepared using 'colour merge' and 'volume viewer' (ImageJ).




N. Belsey et al., J. Control. Release 174 (2014) 37-42

## Controlled skin poration with femtosecond laser pulses




Dye-enhanced, femtosecond pulsed, visible laser radiation, delivered from a hollow core negative curvature fibre, porates mammalian skin.

Ink application to skin surface lowers power required for poration and results in significantly less thermal damage to tissue surrounding pores.

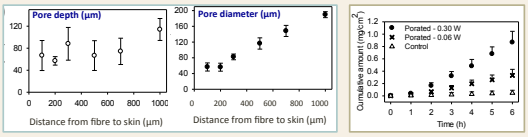
Mechanism attributed to initiation of plasma formation by thermionic electron emission from ink.

H. Garvie-Cook, J.F. Stone, F. Yu, R.H. Guy & S.N. Gordeev, unpublished results.

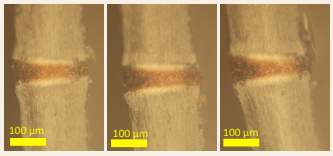
## Controlled skin poration with femtosecond laser pulses

- Ytterbium doped fiber laser (Fianium), wavelength = 1064 nm, pulse duration = 5 ps, repetition rate = 20.
- Pulses compressed and frequency doubled in Li triborate crystal.
- Resulting laser beam had wavelength = 532 nm and pulse duration = ~300 fs. Camera shutter used to expose skin to laser for 1 s.

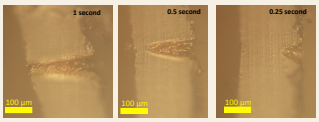
H. Garvie-Cook, J.F. Stone, F. Yu, R.H. Guy & S.N. Gordeev, unpublished results.




## Laser poration of the nail




Cross-sections of inked nails, 130 mW power for 1 s. Reproducible pore formation. 'Colateral' damage apparent.




Decreasing exposure time reduces depth of pores formed. 'Colateral' damage decreased.

## Conclusions

- Novel, non-invasive imaging techniques may (semi-) quantify drug delivery into and through skin.
  - "metamorphosis" of formulations post-application
  - potential to improve topical formulations and optimize drug bioavailability.

- "Large" molecules and objects cannot penetrate an intact skin barrier.
  - nanoparticles as sustained release reservoirs on skin surface, in hair follicles?
  - skin poration approaches – a way forward?
  - what about the "gold standard" of a needle + syringe?