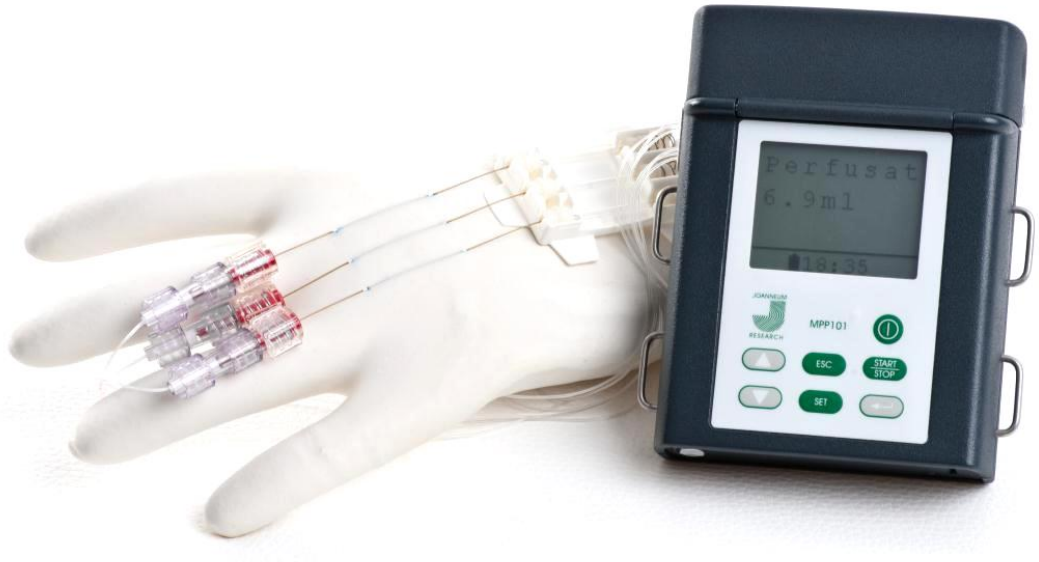
JOANNEUM RESEARCH HEALTH

In-vivo skin PK testing for new and generic topical dermatological drug development



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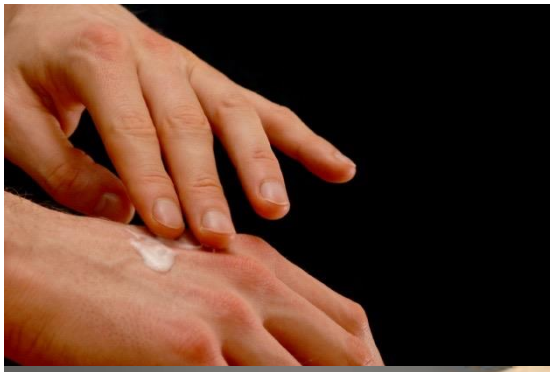
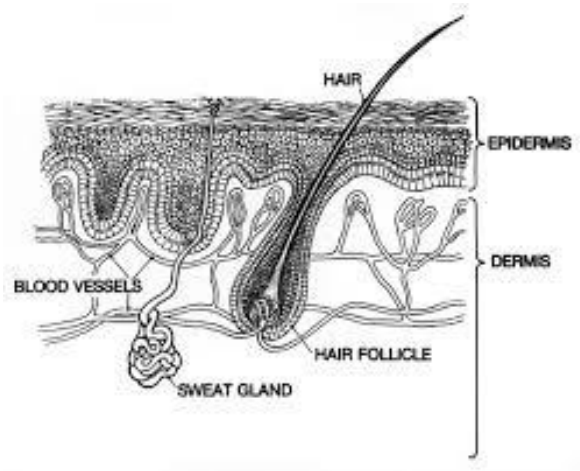
Skin PK approaches
Overview



Does blood really reflect your drug‘s PK/PD in the dermis?



Skin PK approaches
Overview

Skin PK-based approaches are needed for

- Development of locally acting new drugs
  - Proof-of-mechanism
  - PK profile in target tissue
  - Lead compound development
  - Dose-response
  - Formulation optimization
  - Toxicity
  - PK-PD relationship
  - Linking pre-clinical to clinical data
  - …
- Development of locally acting generic drugs
  - PK-based clinical bioequivalence
  - Reduced costs, time and associated development risks

Skin PK approaches
Overview

Suction blister

Target tissue: interstitial fluid

+ quite easy to perform
- no continuous PK profile
- undefined compartment
- possible scar formation

Tape stripping

Target tissue: Stratum Corneum

+ quite easy to perform
+ several time points for PK possible
- no continuous PK profile
- possible scar formation

Biopsies

Target tissue: whole skin

+ quite easy to perform
- no continuous PK profile
- high carry over
- invasive, possible scar formation

RAMAN

Target tissue: SC, Epidermis, Dermis?

+ non-invasive
+ continuous PK profile
- low penetration depth into skin
- expensive instrumentation

Skin PK approaches
Overview

Dermal Microdialysis

Target tissue: Interstitial Fluid

+ continuous PK profile
+ defined compartment
+ minimally invasive
- limited API spectrum due to membrane adsorption and size exclusion
- sampling time limited
- membrane associated effects
- requires standardization
- sensitive analytics needed

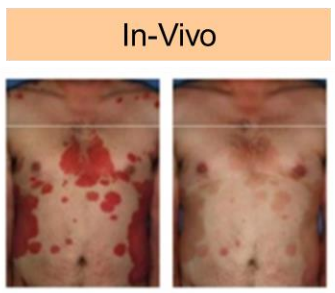
Dermal OFM

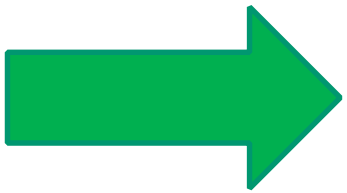
Target tissue: Interstitial Fluid

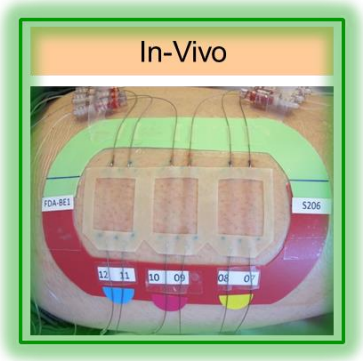
+ continuous PK profile
+ defined compartment
+ minimally invasive
+ entire API spectrum accessible
+ sampling time up to 48 h
- requires standardization
- sensitive analytics needed

Dermal Open Flow Microperfusion
Vision

Vision: open a new way for PK-based bioequivalence studies using dOFM for topical generics







Skin PK-based BE approaches
Overview

Strengths

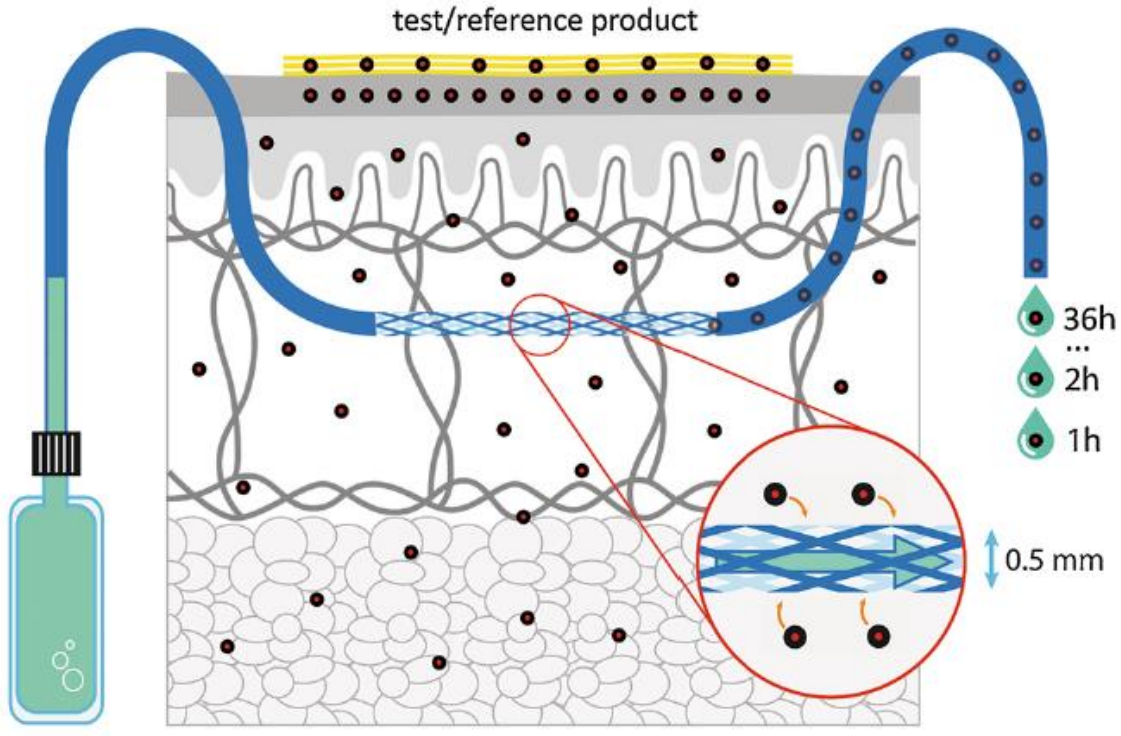
1. Provide a direct in-vivo measurement of the rate and extent of the active moiety at or near the site of action in the dermis.
2. Evidence indicates that dermal sampling has the potential to differentiate pharmacokinetic profiles that correspond to the used concentrations.

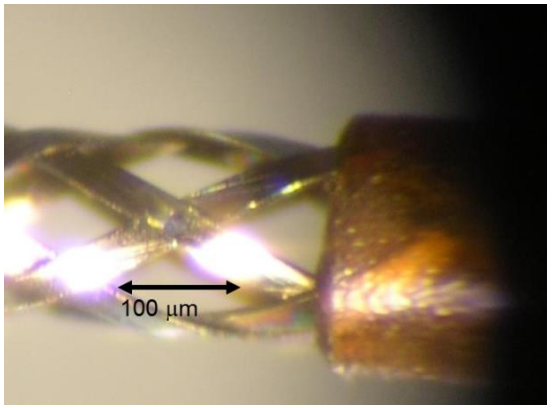
Challenges

1. Robustness of continuous sampling methods
2. Sampling time of 24 hours and more are needed to get ¾ of AUC and C_{max}
3. Highly variable skin penetration

Skin PK-based BE approaches
Open Flow Microperfusion

✓ **OFM samples represent diluted but unfiltered interstitial fluid**

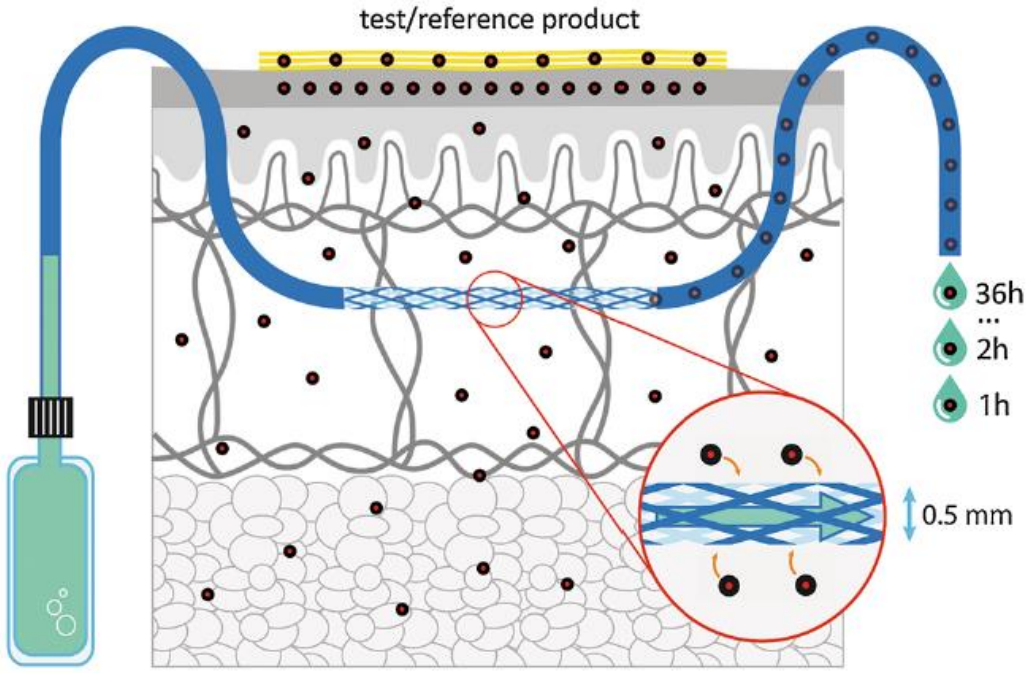




CE-certified for clinical use

Skin PK-based BE approaches
Open Flow Microperfusion

✓ **All drugs are accessible in-vivo in the dermis**



lipophilic substances

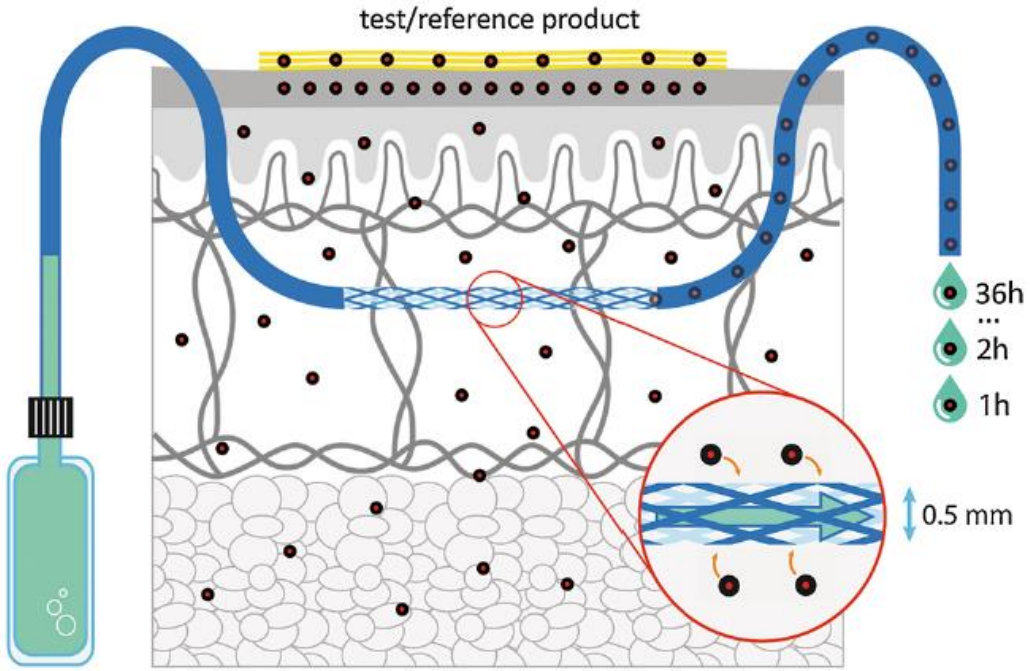
Bodenlenz et al. 2016 (CP-17; logP 3.5)
Holmgaard et al. 2011 (Fentanyl; logP 4.5)

high molecular weight substances (up to cells)

Dragatin et al. 2016
(Quantification of antibodies in skin)

Skin PK-based BE approaches
Open Flow Microperfusion

✓ **dOFM shows dose dependent dermal AUC profiles**

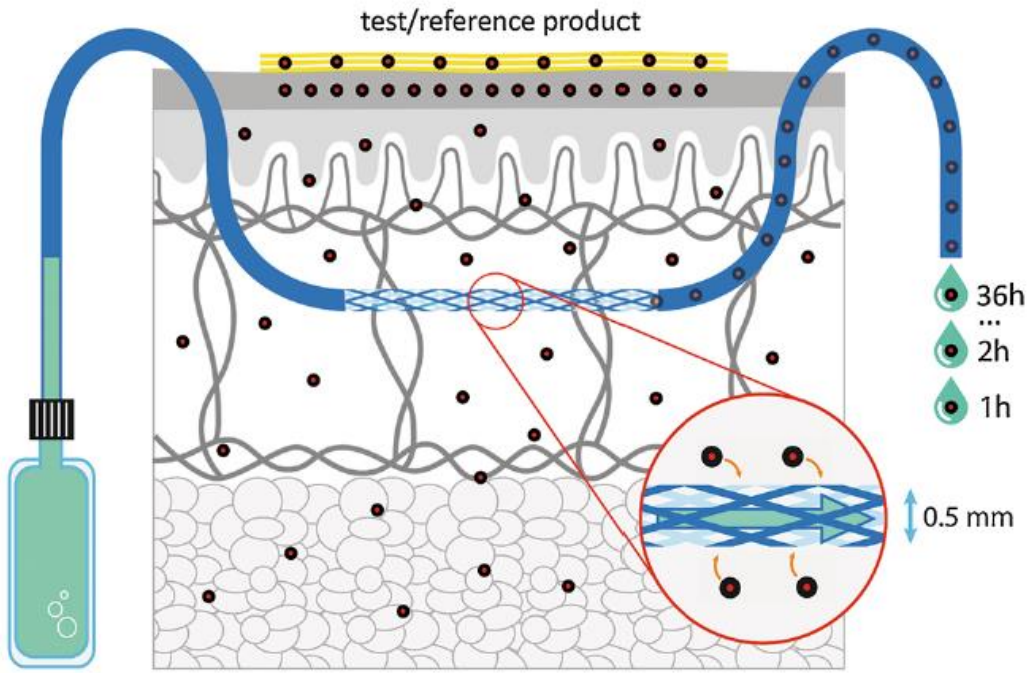


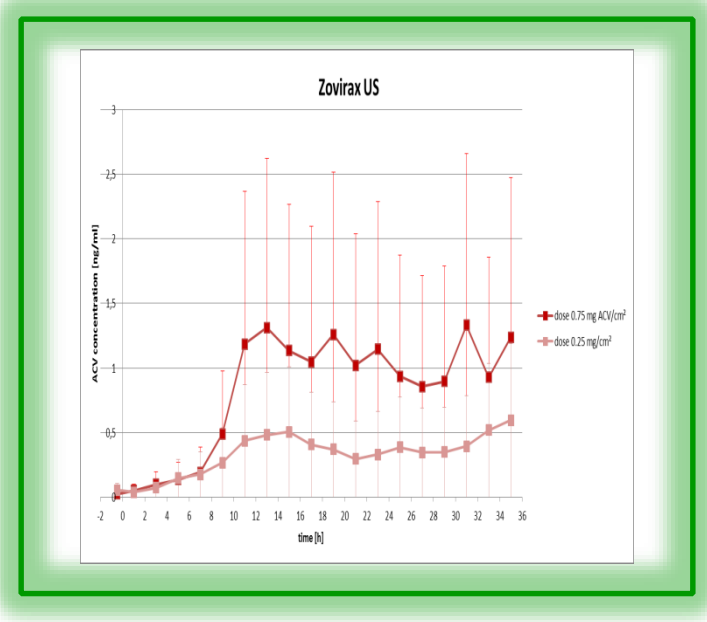
Clinical dOFM studies in skin:

Acyclovir (topical) – 36 h clinical
Corticoid (topical) – 26 h clinical
Antibody (SC) – 17 h clinical

Skin PK-based BE approaches
Open Flow Microperfusion

✓ **dOFM drug concentration is dose dependent**





Skin PK-based BE approaches
Open Flow Microperfusion

✓ **dOFM has a potential for clinical BE studies**

Strengths

1. Provide a direct in-vivo measurement of the rate and extent of the active moiety at or near the site of action in the dermis.
2. Evidence indicates that dermal sampling has the potential to differentiate pharmacokinetic profiles that correspond to the used concentrations.

Challenges

1. Robustness of continuous sampling methods
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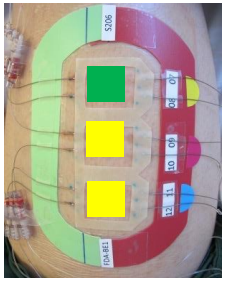
Clinical Bioavailability
Overall Approach

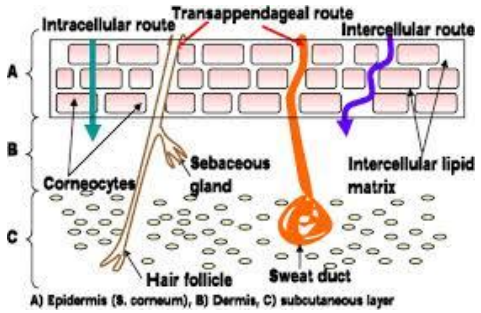
Overall AIM: Investigate the capability of dOFM to address BE and non-BE of topical formulations in-vivo.

- Head-to-Head comparison to minimize inter-subject variability
- Use application-triplets with
  - Two separate application sites for the reference → for BE
  - One application site for a Q1 different drug → for non-BE
- Healthy subjects as a model for the most discriminating study population
- Use of a drug for which skin PK was never successfully monitored in healthy subjects



dOFM
Controlled or Monitored Parameters

✓ **Control for all significant contributing factors adding to data variability - or at least monitor it!**

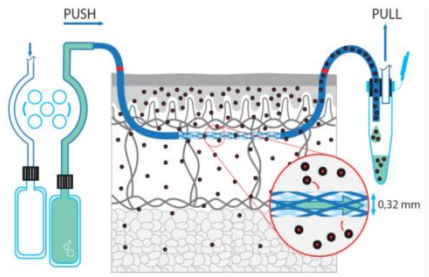


Variations may result from differences in

Hairiness	➔	**not controlled, but counted on photos**
Hair shaving	➔	**subjects are shaved 5 days before dOFM visit**
Sweat duct	➔	not controlled
Skin barrier (stratum corneum) properties	➔	**monitored by TEWL and impedance**
Skin care products use	➔	**not allowed 5 days before dOFM visit**
Skin condition (e.g. Solarium)	➔	**visual check at screening visit**
Room temperature and humidity	➔	**controlled at 22 ± 1° C ; 40 - 60% rel. humidity**

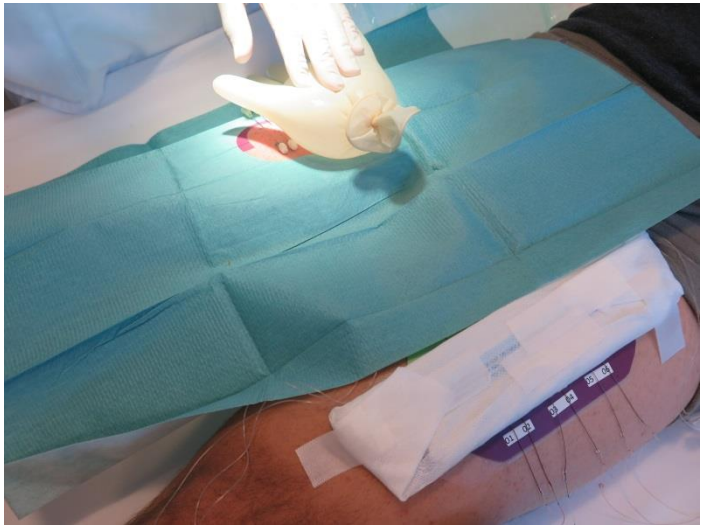
dOFM
Trauma formation

✓ **Minimize trauma formation by cooling**



Variations may result from differences in

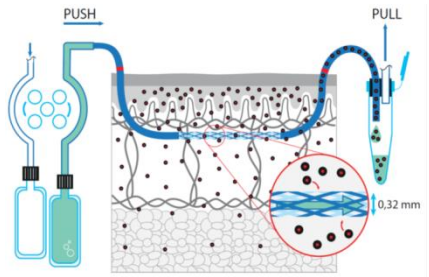
Trauma formation
Application site
Dosage application
Probe depth
Flow rate
Local blood flow
Lateral diffusion and cross-talk
Systemic absorption and cross-talk



Standardized by cooling after dOFM insertion

dOFM
Drug application

✓ **Homogeneous drug application by application template**



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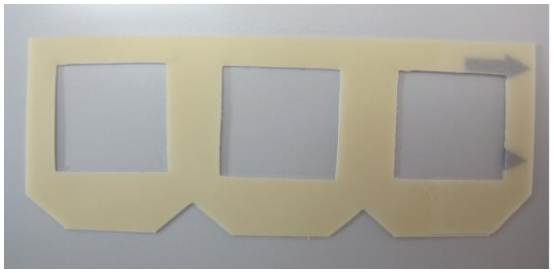
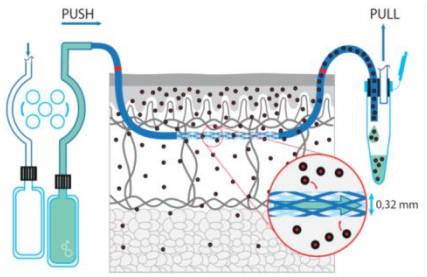


Standardization by use of application template

dOFM
Probe depth

✓ **dOFM probe depth measure for each probe**



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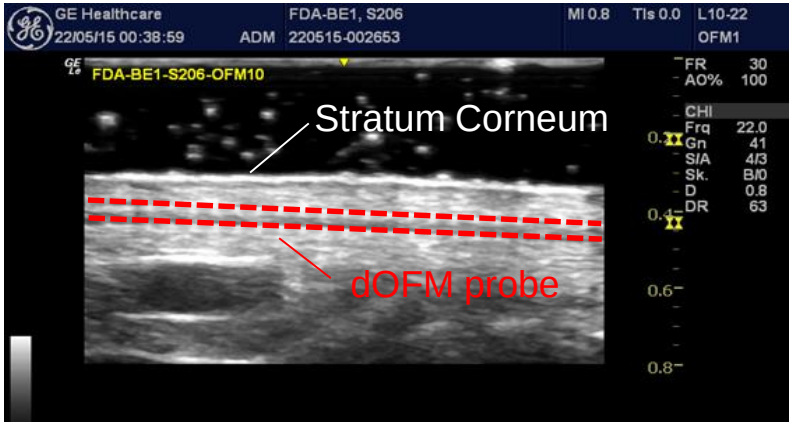
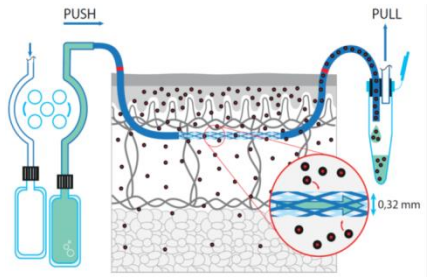


Total exchange area measured by US

dOFM
Flow rate

✓ **Stable flow rate of dOFM probes over 36 hours**



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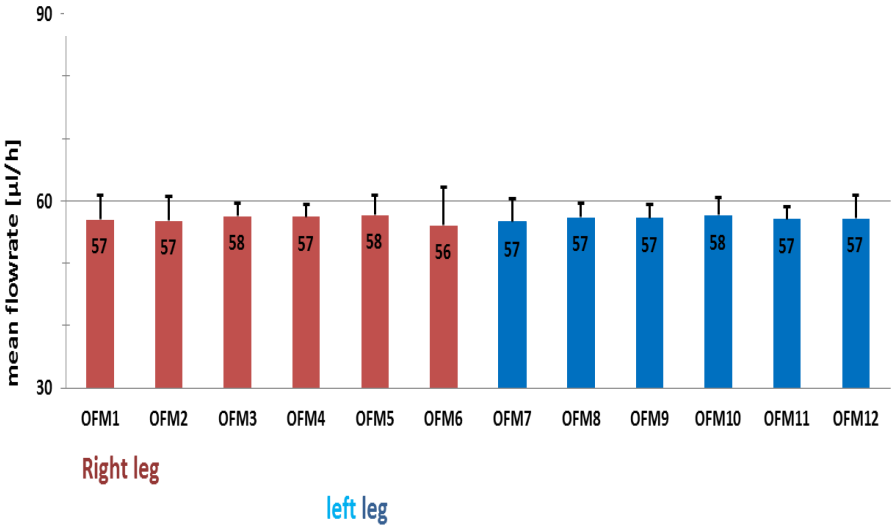
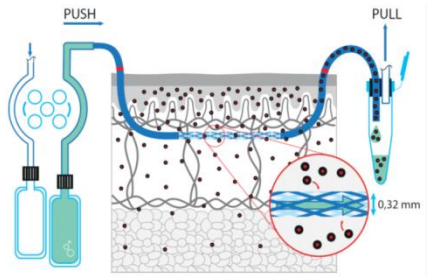


Flow rates of all probes in one subject

dOFM
Local blood flow

✓ **Monitoring of local blood flow by adding internal standard to OFM perfusate**



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Trauma formation
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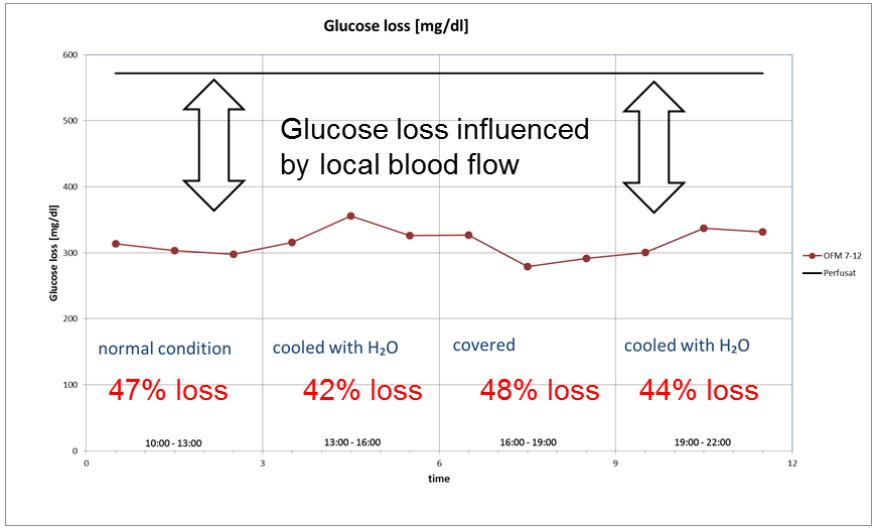


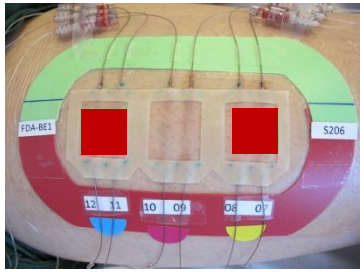
Local blood flow by loss of glucose from dOFM perfusate

dOFM
Systemic adsorption and cross-talk

✓ **No systemic exposure and thus no influence on PK at dOFM site**

Test for Systemic Exposure

- $R = \frac{|\#Blood\ Samples > LLOD|}{|\#Total\ Blood\ Samples|}$
- Definition: no systemic exposure if $R < 0.05$



US Zovirax
Extremely high dose
of 50 mg/cm^2

Methodology

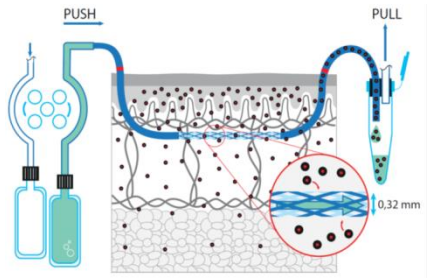
- 6 subjects, 6 application sites
- 10,000 bootstrap estimates were computed
- creation of confidence interval for the true population value of the test statistic R
- a one-sided 95% confidence interval was constructed

Results

MIN	MEDIAN	P90	P95	P99	MAX
0	0.012821	0.025641	0.038462	0.051282	0.064103

dOFM
Controlled or Monitored Parameters

✓ **Highly controlled set-up developed – is a pre-requisite for validated set-up!!**



Variations may result from differences in

Trauma formation	➔	**Controlled by cooling**
Application site	➔	**Controlled by application template**
Dosage application	➔	**Controlled by standardization**
Probe depth	➔	**Monitored by ultrasound**
Flow rate	➔	**Monitored by sample weight**
Local blood flow	➔	**Monitored by glucose marker**
Lateral diffusion and cross-talk	➔	**Negligible**
Systemic absorption and cross-talk	➔	**No systemic exposure**

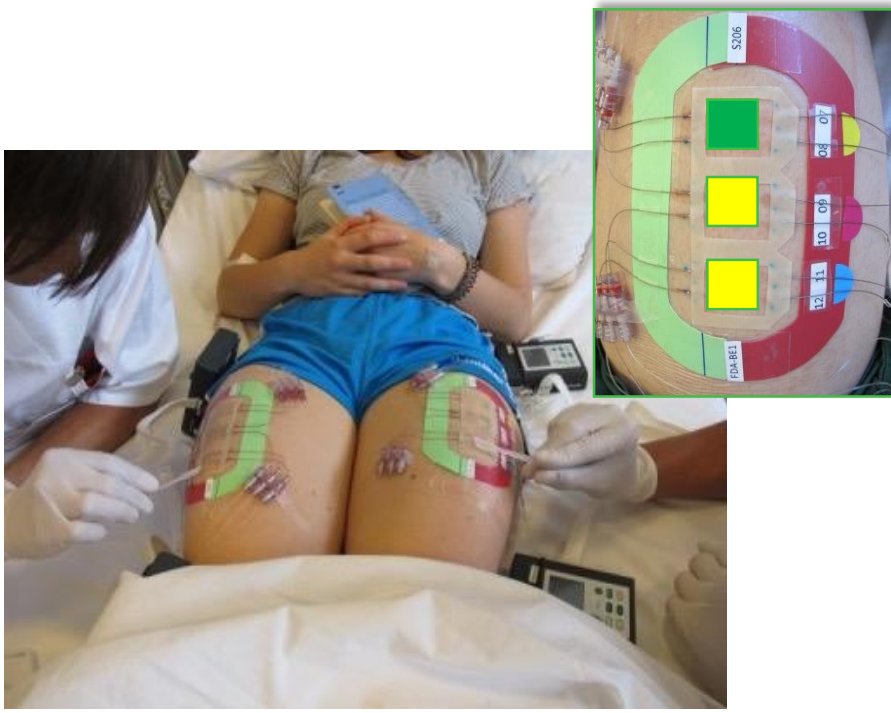
Clinical Bioavailability

Clinical BE Study

Overall AIM: Investigate the capability of dOFM to address BE and non-BE of topical formulations in-vivo.

Overview Clinical Studies:

- 20 healthy subjects
- Reference: Zovirax ® US
- Test: Aciclovir-A1 Pharma Austria
- 2 application-triplets per subject
- 15 mg/cm^2 drug application
- 36 hours dOFM sampling time



dOFM
Clinical Study Details

- ✓ **Test and Reference are both 5% acyclovir creams but NON-Q1**
- ✓ **IVRT: identical release R:R and non identical release T:R**

Zovirax (R) (USA)	Aciclovir-1A (Austria)
Water	Water
Propylene glycol	Propylene glycol
Mineral oil	Viscous Paraffin
White petrolatum	White Vaseline
Cetostearyl alcohol	Cetyl alcohol
SLS	Not disclosed
Poloxamer 407	Not disclosed
Not disclosed	Dimethicone
Not disclosed	Glyceryl Mono Stearate
Not disclosed	Polyoxyethylene stearate

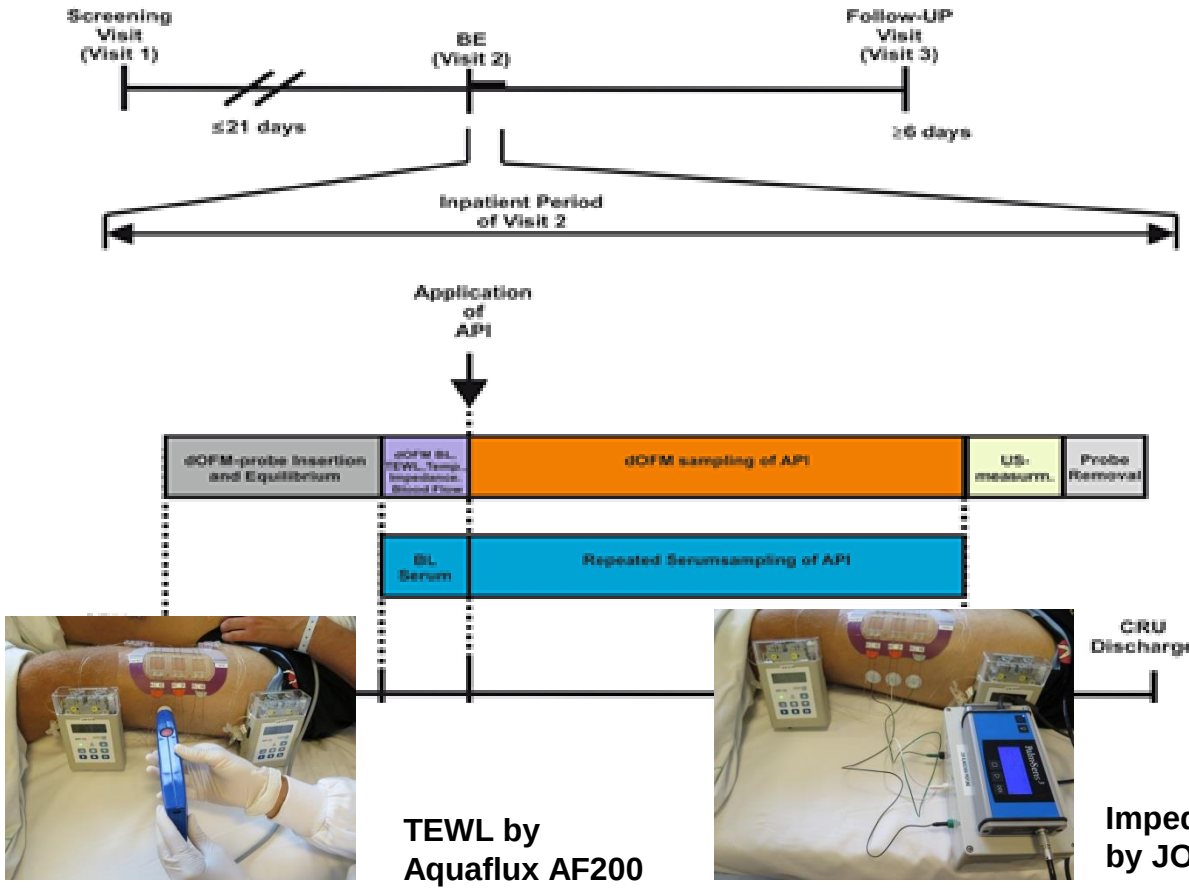
Non-Q1

Equivalence comparison	Computed confidence interval	
	Lower Limit	Upper Limit
Zovirax cream 5% US v. Zovirax cream 5% US	85.7	103.02
Zovirax cream 5% US v. Aciclovir 1A Pharma Cream 5%	16.27	19.60

Acceptance limits: [75%, 133.33%]

dOFM
Clinical Study Details

✓ **Clinical BE study design**



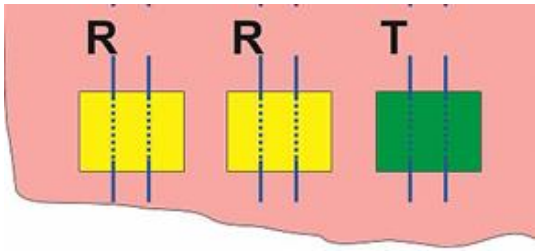
TEWL by Aquaflux AF200

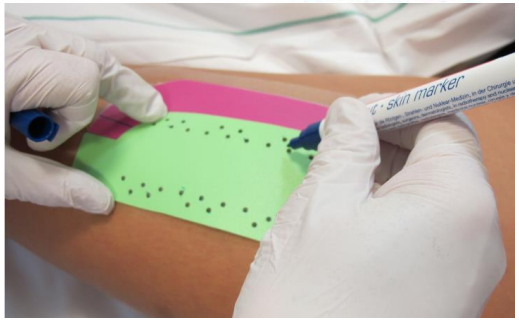
Impedance by JOANNEUM

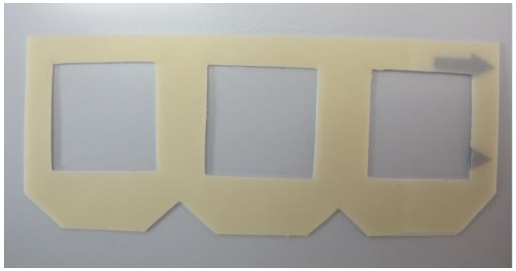
Ultrasound GE-Healthcare

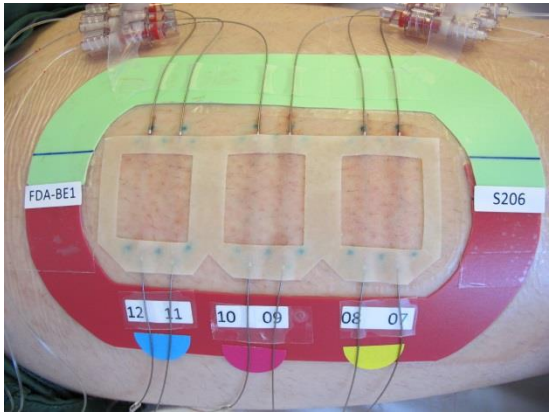
Clinical Bioavailability
Clinical BE Study

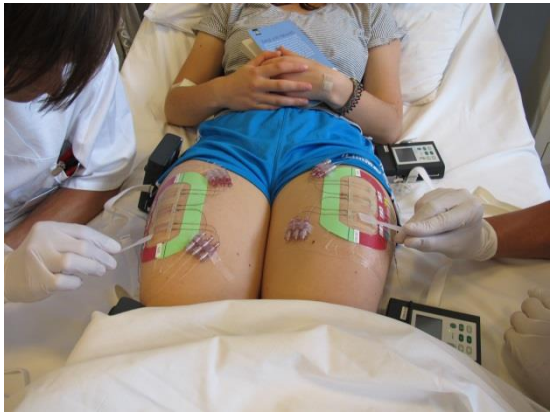
✓ **All procedures are standardized by using templates and SOPs**

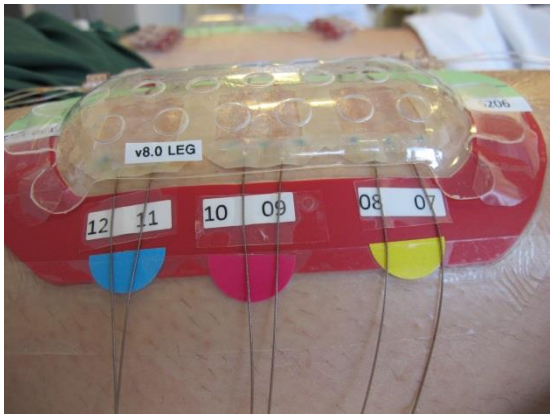


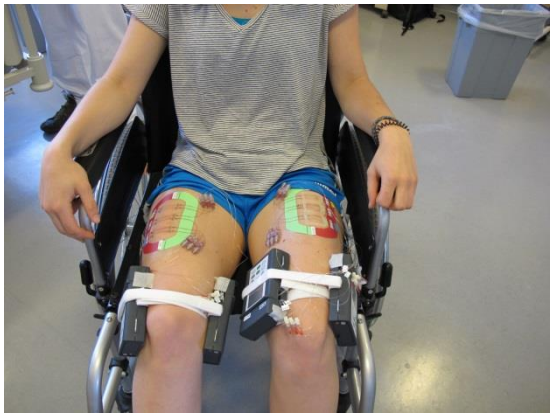


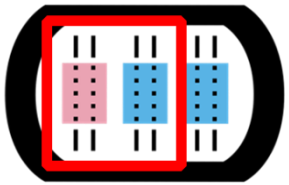






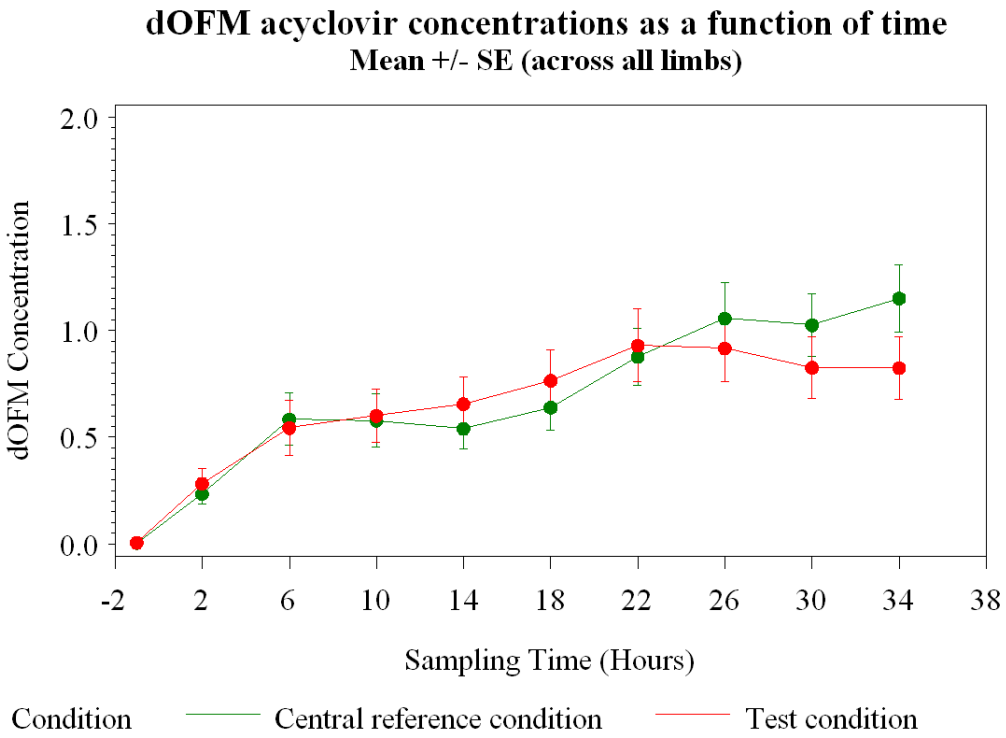




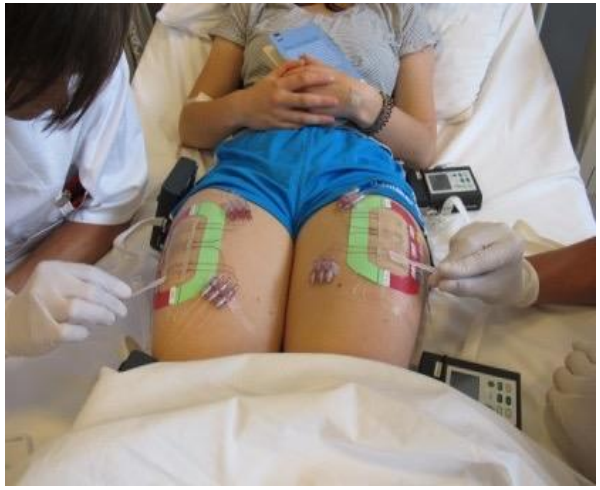


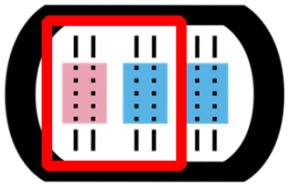
Clinical Bioavailability
Test versus Reference

✓ **Bioavailability: AUC and T_{max} of Aciclovir A1 are highly reproducible**
AUC and T_{max} of Zovirax US are highly reproducible



20 healthy subjects





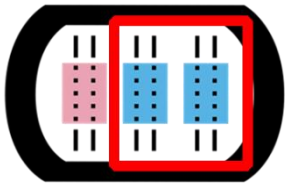
Clinical Bioavailability
Test versus Reference

- ✓ **BA is different for Aciclovir 1A vs Zovirax US based on AUC**
- ✓ **BA is different for Aciclovir 1A vs Zovirax US based on C_{max}**

Outcome variable	$CI_{90\%}$	BE-limits	$CI_{90\%}$ within BE-limits
log(AUC0-36h)	[-0.369 ; 0.050] or [69.1 % ; 105.2 %]	[-0.223 ; 0.223] or [80% ; 125%]	x Failed
log(C_{max})	[-0.498 ; 0.022] or [60.8 % ; 102.2%]		x Failed

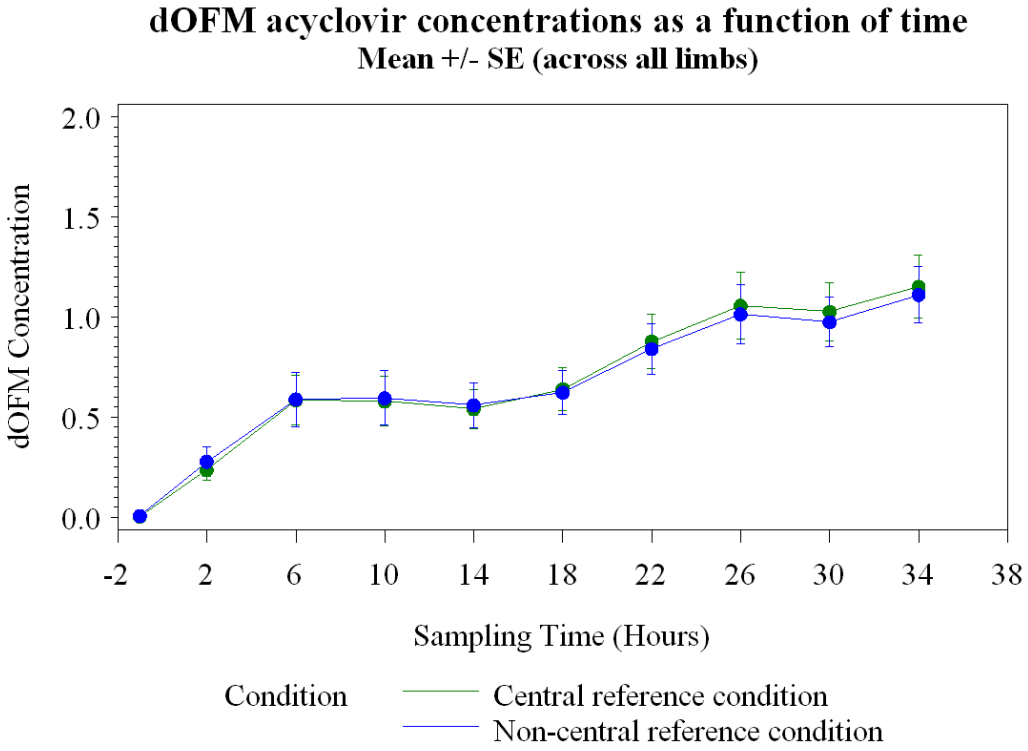
BA is tested for the difference of the log-transformed outcome variables (AUC, C_{max}) between test and reference condition

BA is established if $CI_{90\%}$ falls within the limits of log(0.8)=-0.223 and log(1.25)=0.223 (cf. FDA Guidance For Industry)

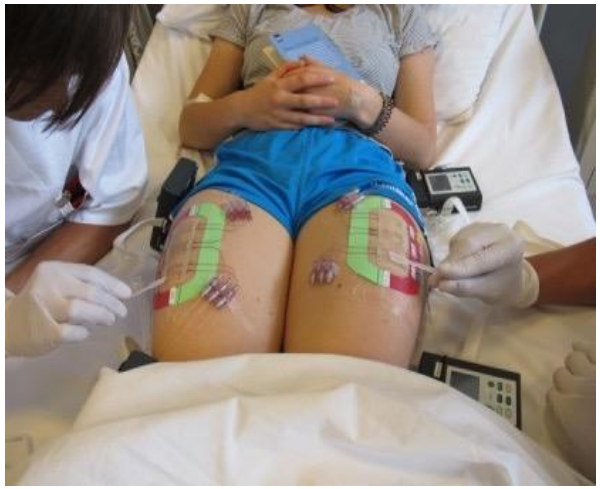


Clinical Bioavailability
Reference versus Reference

✓ **Bioavailability: AUC and C_{max} of Zovirax US are highly reproducible**



20 healthy subjects



"Open Flow Microperfusion as a Dermal Pharmacokinetic Approach to Evaluate Topical Bioequivalence"
Clin. Pharmacokinet. 8/2016 – OPEN ACCESS

Clinical Bioavailability
Reference versus Reference

- ✓ **Same BA for Zovirax US vs Zovirax US based on AUC**
- ✓ **Same BA for Zovirax US vs Zovirax US based on C_{max}**

Outcome variable	$CI_{90\%}$	BE-limits	$CI_{90\%}$ within BE-limits
log(AUC0-36h)	[-0.148 ; 0.162] or [86.2 % ; 117.5 %]	[-0.223 ; 0.223] or [80% ; 125%]	**passed**
log(C_{max})	[-0.155 ; 0.190] or [85.7 % ; 120.9%]		**passed**

BA is tested for the difference of the log-transformed outcome variables (AUC, C_{max}) between the two reference conditions

BA is established if $CI_{90\%}$ falls within the limits of log(0.8) = -0.223 and log(1.25) = 0.223 (cf. FDA Guidance For Industry)

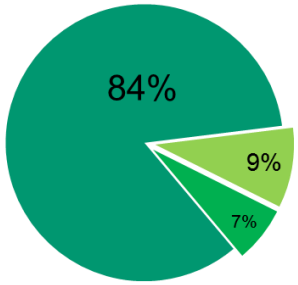
Skin penetration insights
Total variability

✓ **dOFM has a low total and intra-subject variability**

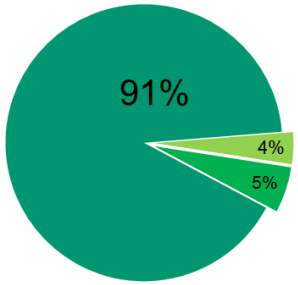
Total $CV_{logAUCacyc}$ was **39-44%** (40-93% Microdialysis Benfeld et al.)

Total variability (ANOVA)

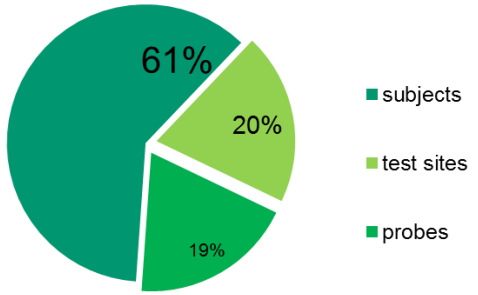
- Inter-subject variability: 84-91% OFM (61% Microdialysis Benfeld et al.)
- Intra-subject variability: 9-16% OFM (39% Microdialysis Benfeld et al.)



logAUC Zovirax®



logAUC Aciclovir A1 Pharma



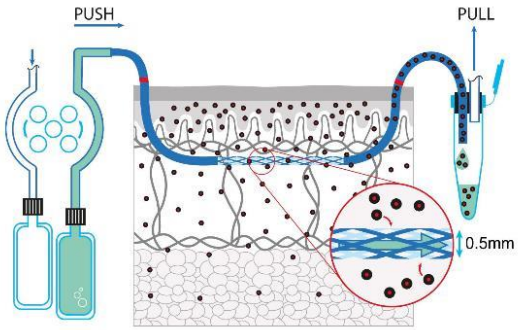
logAUC lidocaine MD (Benfeld et al.)

Benfeldt et, J Invest Dermatol. 2007 Jan;127(1):170-8. Epub 2006 Jul 27

Ex-vivo BE

Repetition of the in-vivo dOFM BE study in ex-vivo skin

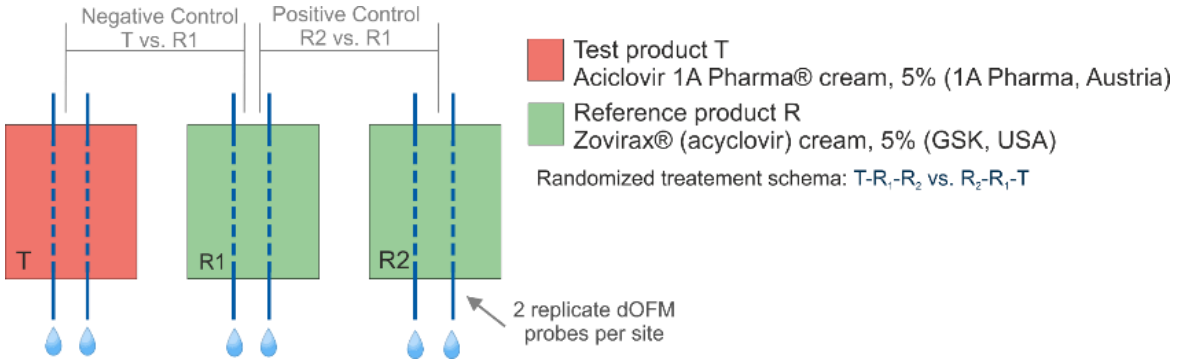
- Investigate the usability of open flow microperfusion (OFM) for bioequivalence (BE)
  testing of topically applied drugs in excised human skin explants

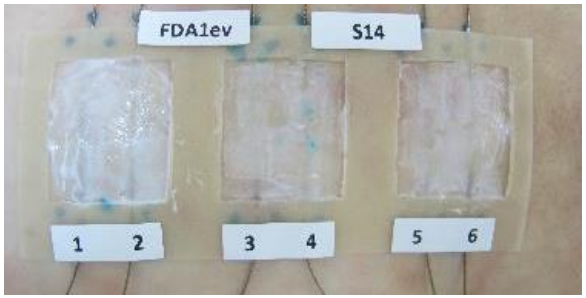


- Measure dermal concentrations of two acyclovir products to assess their PK endpoints AUC and C_{MAX}
- BE evaluations - using the average BE (ABE) and reference-scaled (SABE) statistical approach for following comparisons:
  a) Positive control: Reference product against itself
  b) Negative control: Reference product against a non-equivalent test product

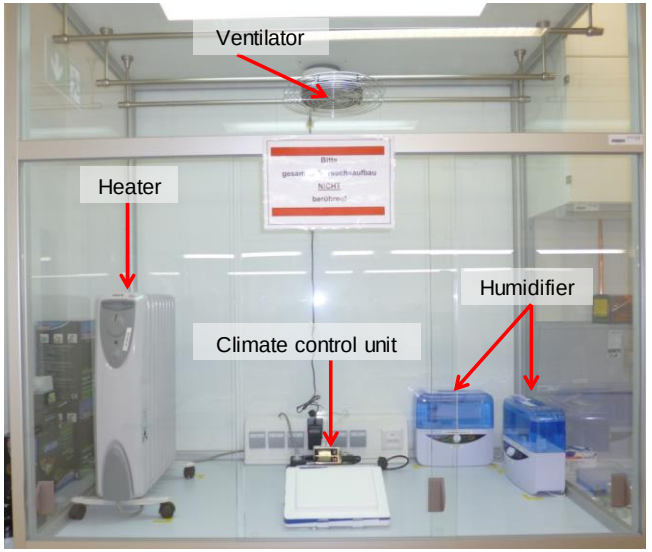
Ex-vivo BE
Study design

- 40 full-thickness human skin explants (16 donors)
- Topical application of two 5% acyclovir cream (15 mg/cm²):

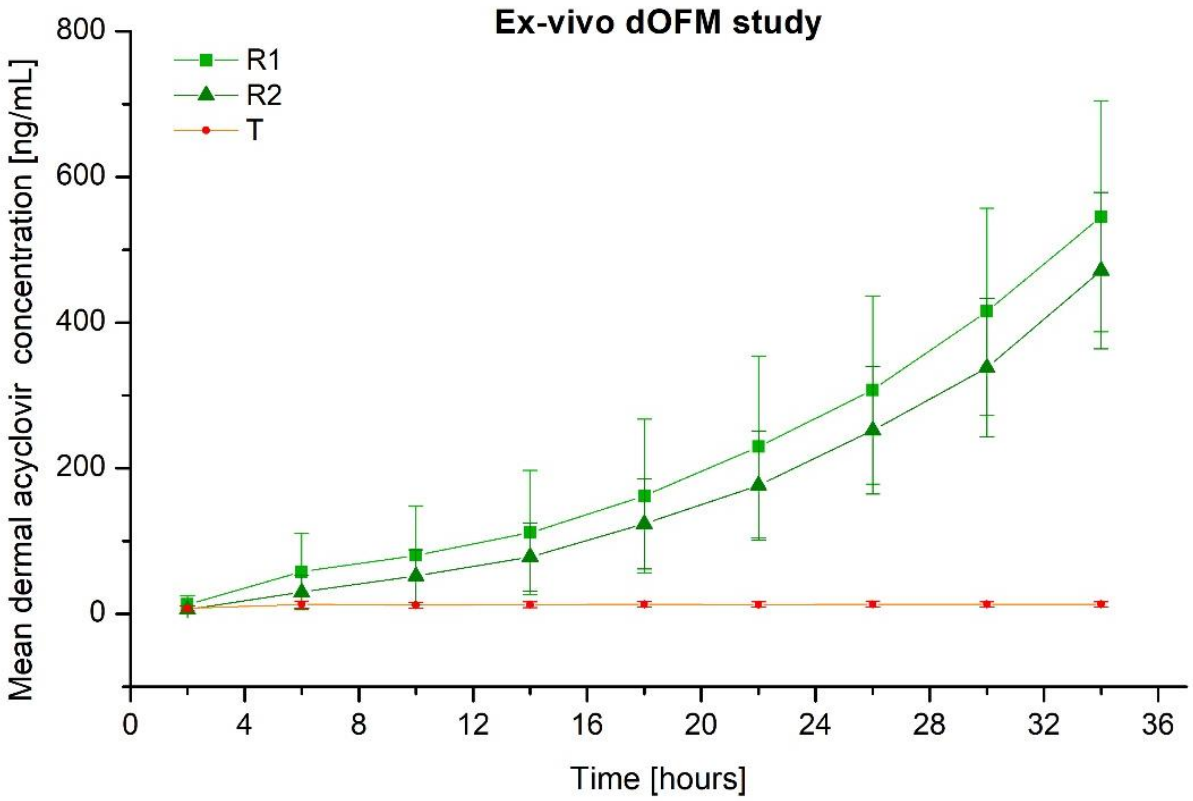


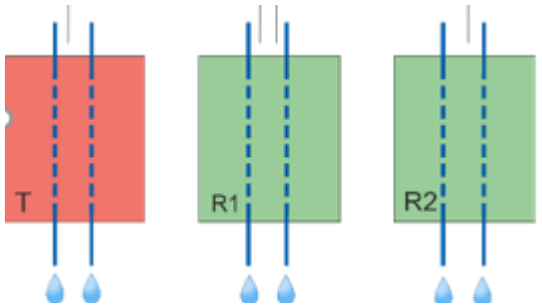


- Continuous ISF sampling
  - T= -1–0 h: Baseline sampling from -1 - 0 h
  - T= 0 h: Topical application
  - T= 0–36 h: Post dose sampling in 4 h intervals
- Controlled environmental conditions: 22±1°C, 40-60% RH
- Bioanalytical method: UHPLC-MS for quantification of acyclovir in ISF samples



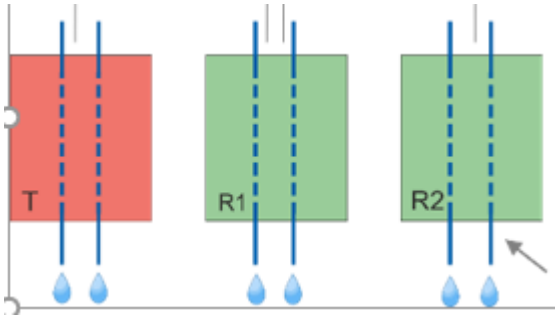
Ex-vivo BE
Concentration-time profiles





Ex-vivo BE
BE Evaluations

SABE	PK endpoint	S_{WR}	Upper 95% bound of the scaled CI	GMR	Passed
Postive control (R1 vs. R2)	AUC_{0-36h}	0.68	-0.159	1.1771	✓
	C_{max}	0.60	-0.094	1.1918	
Negative control (T vs.R1)	AUC_{0-36h}	0.68	8.989	0.0764	x
	C_{max}	0.60	16.050	0.0293	



Pharmacokinetics-Based dOFM
Summary

dOFM in-vivo

- Is a reproducible, accurate and sensitive method
- Shows very low method-variability
- Reflects in-vivo skin penetration in dermis
- Gives advanced skin penetration insights
- Is able to investigate BE on a dermato-pharmacokinetic basis

➔ **BE OFM set-up will be further optimized to a universal dermato-pharmacokinetic-based BE approach for topical drugs by carrying out more clinical studies**

dOFM Bioequivalence
Outlook

dOFM Study 1: Moderate Lipophilic/Protein Bound Drugs (low amount, non-occlusive, infinite dose)

- Pilot: Assess parameters for the design of the subsequent BE study (n=6) ✓
- Main study: Identify influencing factors (n=20) – planned spring 2019

dOFM BE Study 2: Moderate Lipophilic/Protein Bound Drugs (high amount, occlusive, finite dose)

- Pilot: Assess parameters for the design of the subsequent study (n=6) ✓
- Clearance study: assess systemic drug clearance (n=6) – planned summer 2019
- Main study: Identify influencing factors (n=20) – planned summer 2019

dOFM BE Study 3: Highly Protein Bound Drug

- Pilot: Assess parameters for the design of the subsequent study (n=6) – planned for autumn 2019
- Main study: BE study (n=20) – planned for winter 2019

➔ Show the potential of OFM as a universal tool for BE Studies for topical drugs

A big thanks to…

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Statistics

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Jill Coker
and other….

Thank you for your attention

Dr. Frank Sinner
JOANNEUM RESEARCH Forschungsgesellschaft mbH
HEALTH – Institute for Biomedicine and Health Sciences
Neue Stiftingtalstrasse 2, 8010 Graz
+43 316 876-4000
frank.sinner@joanneum.at
www.joanneum.at/health