The Development and Application of Surrogate Methods to Assess Bioequivalence of Topical Generic Products Intended for Local Action

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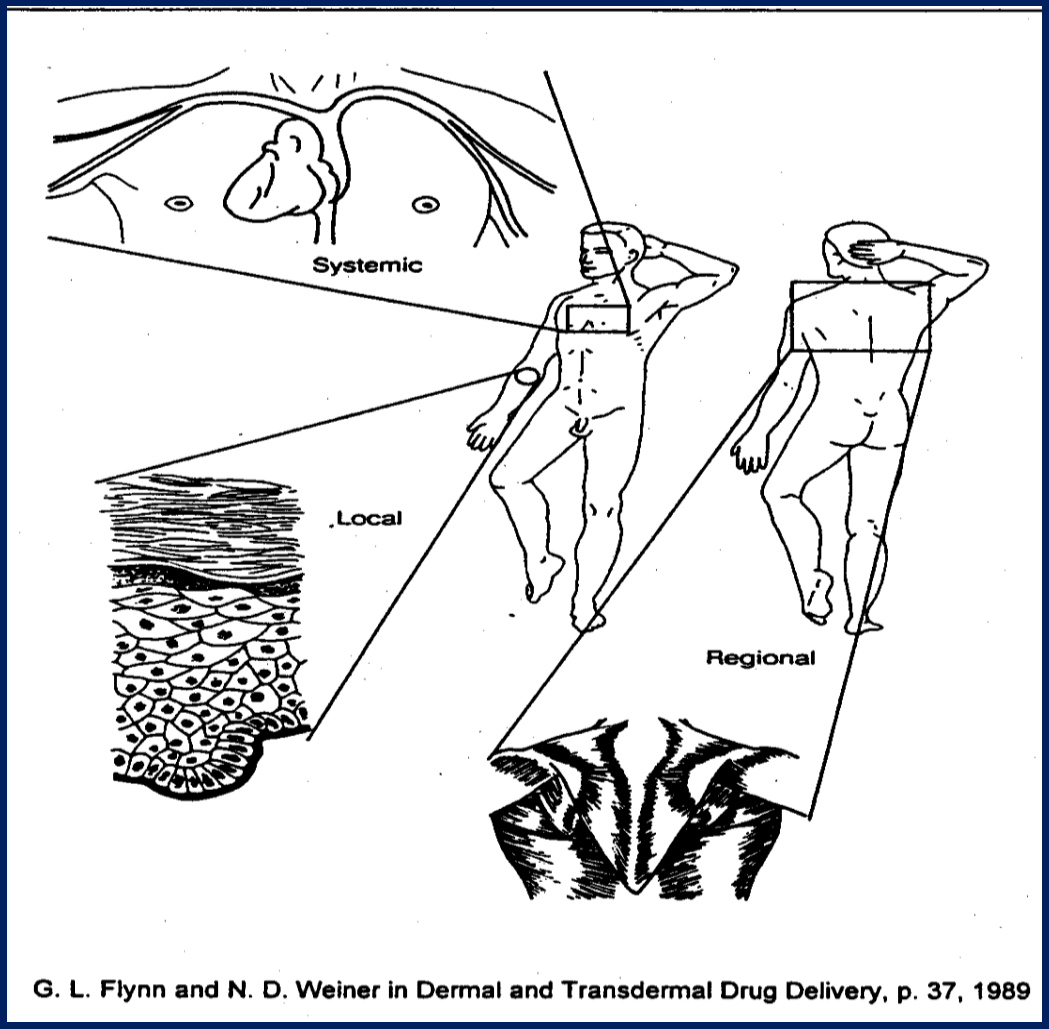
- **Undoubtedly, the major focus of attention for the assessment of bioequivalence of drug products has been in the area of drugs administered extravascularly and intended to be absorbed into the systemic circulation.**

- **Approach is longstanding and involves the measurement of drug concentration in the blood following administration of a specific extravascular dosage form.**

- **NON-ABSORBED drugs, i.e. Topical dosage forms intended for local action**



G. L. Flynn and N. D. Weiner in Dermal and Transdermal Drug Delivery, p. 37, 1989

TOPICAL

“Belonging to a Place or Spot”

Topical = Local

So, what approach can be used for the BE assessment of topical products ?

a) Topical products for systemic action

e.g. transdermal dosage forms and semi-solid or liquid dosage forms applied to the external surface of the body – nitroglycerine patch or anti-inflammatory agents for *regional* action?

b) Topical products for local action only

i.e not intended to be absorbed into the systemic circulation and also applied to the external surface of the body – anti-fungal agents?

TOPICAL
“Belonging to a Place or Spot”
Topical = Local

- TRANSDERMAL products for treatment of systemic diseases

Aimed at achieving active drug concentrations in the systemic circulation

Percutaneous absorption is a prerequisite for activity

Ideally, no local drug accumulation

TOPICAL
“Belonging to a Place or Spot”
Topical = Local

- TOPICAL products for cutaneous/local (dermatologic) use

  Pharmacologic or other effect confined to surface of skin or within the skin

  May or may not require percutaneous penetration and deposition

TOPICAL
"Belonging to a Place or Spot"
Topical = Local

- **REGIONAL products** for treatment of disease or symptoms in deeper tissue

Pharmcological action effected within musculature, vasculature, joints, synovial fluid beneath and around application site

More selective activity compared to systemic delivery

Requires percutaneous absorption and deposition

BIOAVAILABILITY

- For systemically absorbed products it is:

..... the rate and extent to which the active ingredient or moiety is absorbed from the drug product and becomes available at the site of action.

- For products **not intended to be absorbed** into the systemic circulation - ? → RELEASE!

This is an issue of "RELEASE" from the dosage form!

Surrogate Methods

- **Surrogate measures justified by the presumption that concentration of drug in blood stream is in equilibrium and reflects the concentration at site of action**

- **Relationship between effectiveness and systemic blood concentrations of drug implied**

- **Measurements of drug concentrations in blood following application of a topical dosage form intended for local action only cannot be justified on the same basis as for drugs intended to be absorbed**

- **Although some drug may enter the systemic circulation, a direct relationship between effectiveness and systemic blood concentrations is highly dubious and unlikely**

Products Intended to be Absorbed into Systemic Circulation

- Methodology well established
- Statistical assessment of data well established
- Regulatory requirements based up C_{max} and AUC falling within prescribed limits of CI of 90% and relative means of test to reference being within 80-125%

Topical Products Not Intended to be Absorbed

- Methodology under development
- Statistical assessment yet to be defined
- Regulatory requirements?

CURRENT AVAILABLE METHODS FOR THE ASSESSMENT OF BE OF TOPICAL DOSAGE FORMS FOR LOCAL ACTION

a) Clinical end point studies to assess efficacy and safety

b) The **Human Skin Blanching Assay** (HBSA) *aka* the Vasoconstrictor Assay (VCA) for assessing topical products containing topical corticosteroids ONLY!

THAT's IT!

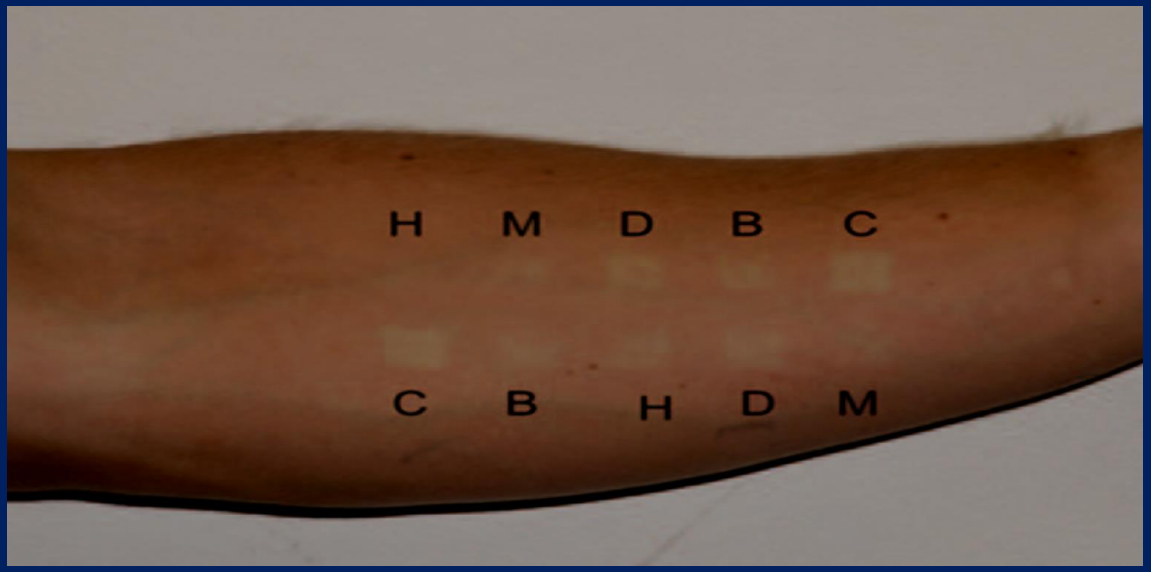
WELL-ESTABLISHED METHODS

The **Human Skin Blanching Assay** (HBSA) *aka* the Vasoconstrictor Assay (VCA) for assessing topical corticosteroid products

First observed in 1950 (Hollander *et al*)

McKenzie & Stoughton – 1962

Indirect measure using a supposed vasoconstriction response following application of topical corticosteroid to skin



H = hydrocortisone 17-butyrate cream (1 mg/ gm), C = clobetasol propionate cream (0.05 %), B = betamethasone valerate cream (5 mg/5 gm), D = desoximetasone ointment (0.25 %), and M = mometasone furoate cream (0.1 %)

Ref:

Methods for the Assessment of Bioequivalence of Topical Dosage Forms: Correlations, Optimization Strategies and Innovative Approaches
Isadore Kanfer
In " Topical Drug Bioavailability, Bioequivalence and Penetration" 2nd Edition,
Edited by John Jenner, Vinod P Shah & Howard I Maibach
Chapter 31, Springer Science, New York, 2014

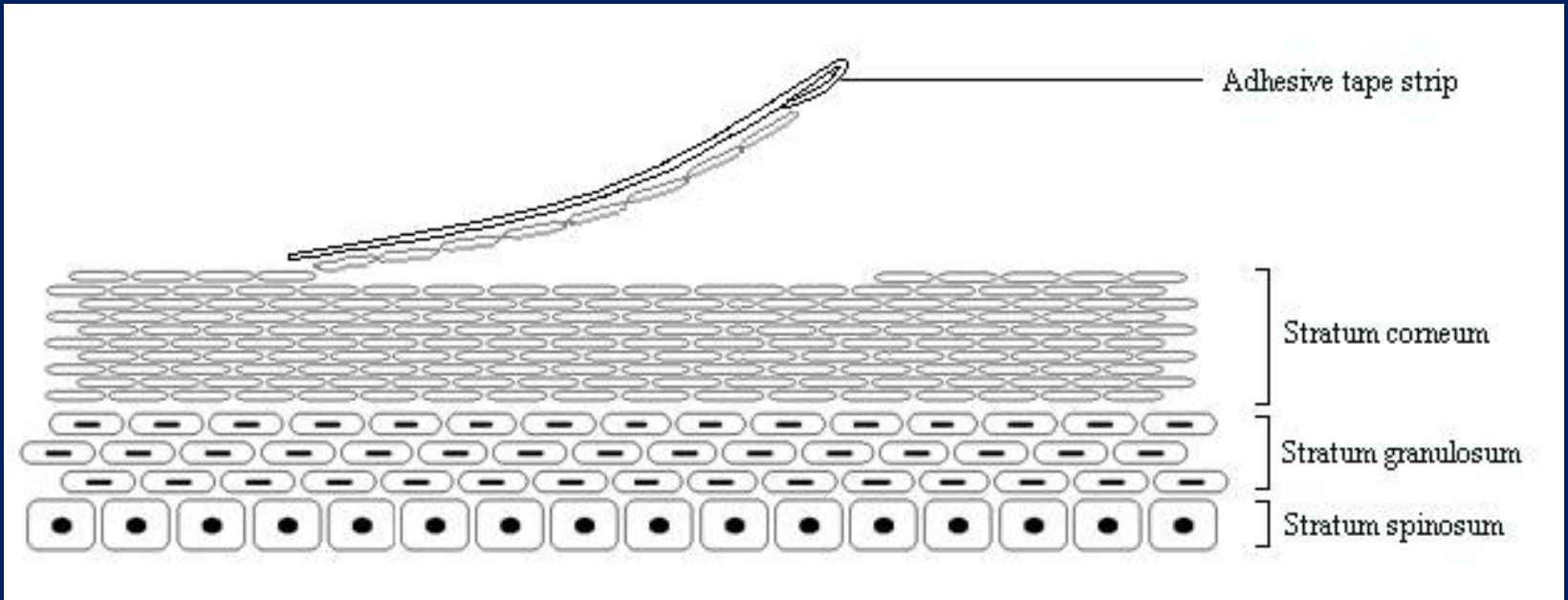
Innovative and other methodologies currently under investigation for the assessment of BA/BE of topical products for local action

- **Dermatopharmacokinetic methods – Tape Stripping**
- **Dermal microdialysis (DMD)**
- **Dermal open flow micro-diffusion (dOFMD)**
- ***In vitro* release/diffusion methods**

Tape Stripping – a dermatopharmacokinetic approach

- U.S Food and Drug Administration (FDA). 1998. Guidance for Industry, Topical Dermatological Drug Product NDAs and ANDAs - *In Vivo* Bioavailability, Bioequivalence, *In Vitro* Release and Associated Studies
- Initial TS methodology outlining the bioavailability/bioequivalence protocol for topical formulations intended for local and/or regional activity, published in a draft guideline
- Subject to criticism which resulted in its withdrawal, mainly due to a number of limitations, in particular the sources of variability and control

- Dermatopharmacokinetic approach
- Determines the amount of drug permeated into the *stratum corneum*
- Utilizes adhesive tape strips Transpore, Micropore, Scotch, D-Squame tapes
- Relatively non-invasive
- Removes layers of *stratum corneum*


Adhesive tape strip
Stratum corneum
Stratum granulosum
Stratum spinosum

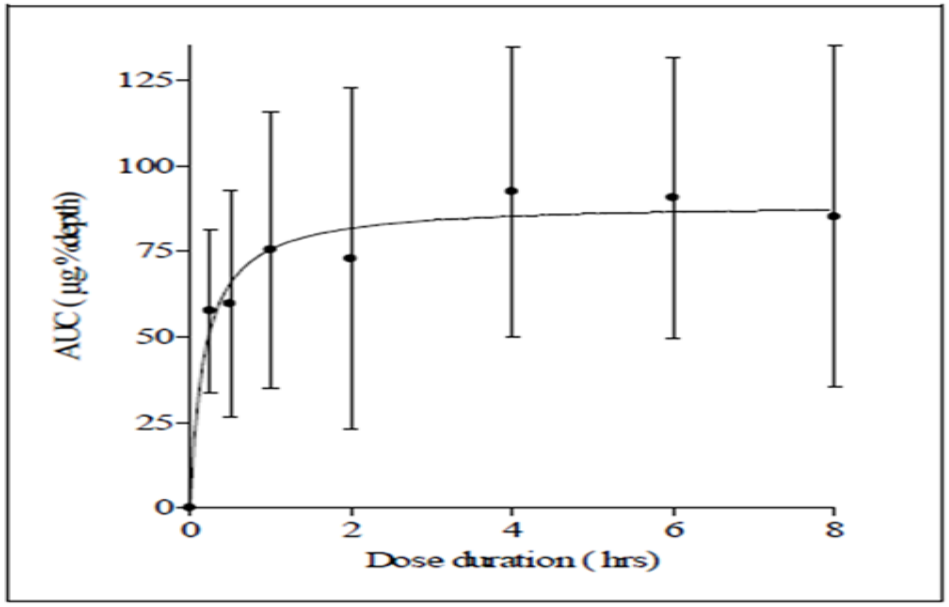
How long should the dose be left on the application sites prior to skin stripping?

- The choice of dose duration has generally been unsubstantiated.
- Sampling when the concentration of drug in the SC is at steady state is likely to mask differences in formulations, thus it is important to have a validated method of ensuring that the chosen dose duration falls on a sensitive part of a dose – response relationship, such as a plot of the dose duration *vs*. drug penetration profile.
- In order to determine a dose duration which will provide the necessary discriminatory power to identify significant differences or equality between products, the approach employed in the FDA HSBA guidance was used.

Determination of ED_{50}

- Perform a pilot study and use the E_{max} model to determine the dose duration where the maximum sensitivity can be expected – i.e. the ED_{50}.

i.e. carried out at the most sensitive part of the dose-response curve



Dose duration profile for Canesten® Topical cream (n=10).

The data were fitted to the E_{max} model with R^2 = 0.9648, E_{max} = 89.06 and ED_{50} = 0.801 or 10.8 mins

What about differences in skin thickness between subjects?

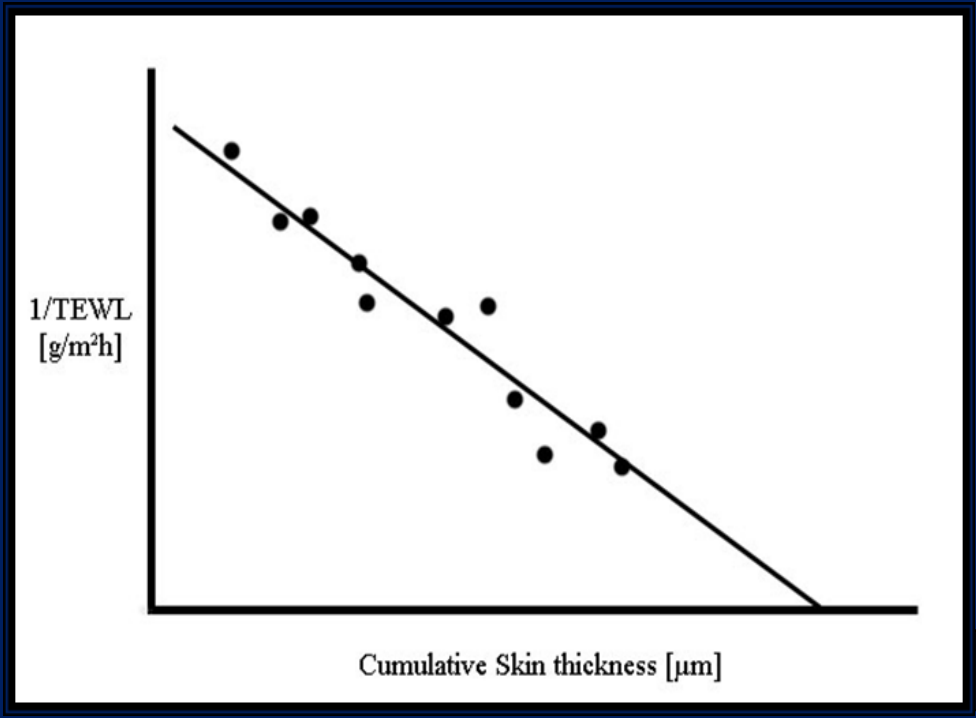
How can such skin thickness differences be normalized ?

Transepidermal water loss (TEWL) measurements

- TEWL measurements were taken at the blank site only and used to determine *SC* thickness/subject
- *Stratum corneum* thickness differs between individuals – hence, normalization necessary - measure transepidermal water loss (TEWL):

$1/J = 1/TEWL_x = H\text{-}x/K.D.\Delta C$[1]

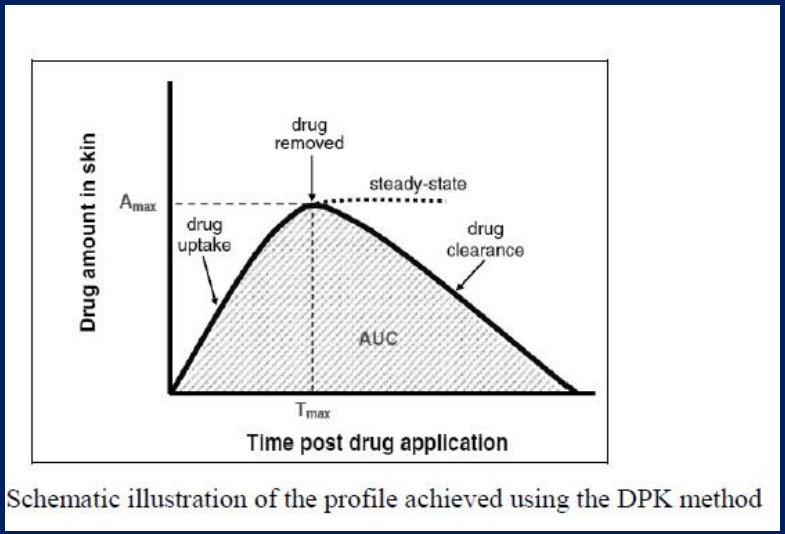
- H can be determined by the x-intercept of the plot 1/TEWLx vs. x.



J = flux g/m^2h
H = total SC thickness
x = partial SC thickness
K = partition coefficient of water in tissue
D = water diffusivity
ΔC = difference in water concentration across the membrane

Kalia *et al*, Pharm. Res.17,1148-1150, 2000

Appropriateness of using parameters such as AUC and Amax - parameters derived from the principles of oral pharmacokinetics ?



Schematic illustration of the profile achieved using the DPK method

- After topical application of a drug, concentration found at the "site of action" is determined primarily by **SC penetration and processes such as partitioning, diffusion and keratin binding**.
- In contrast, when using the oral route, the plasma concentration *vs.* time profile obtained is controlled by the **processes of absorption, distribution, metabolism and elimination.**

Case Studies

Conduct 2 biostudies to confirm that the TS method can discriminate between:

- *bioequivalent* topical creams containing 5 % acyclovir
- a *bioinequivalent* topical cream containing 1.5% acyclovir

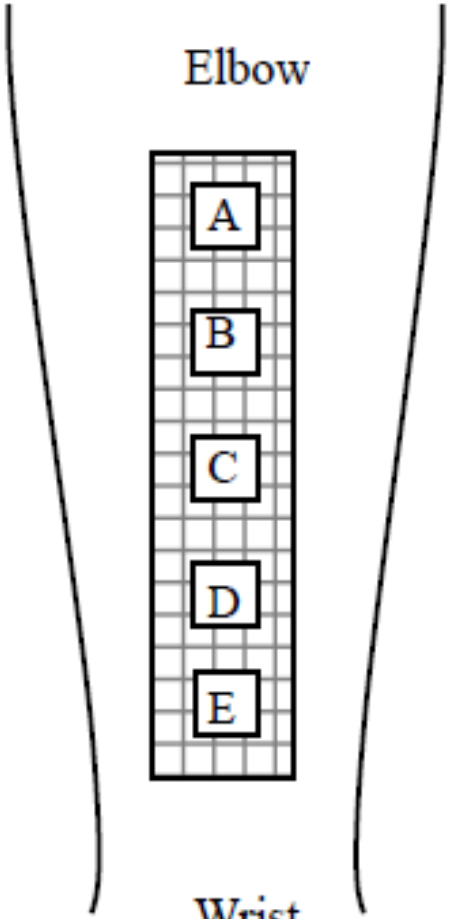
Bioequivalence of Topical Formulations containing 5% acyclovir using Tape Stripping

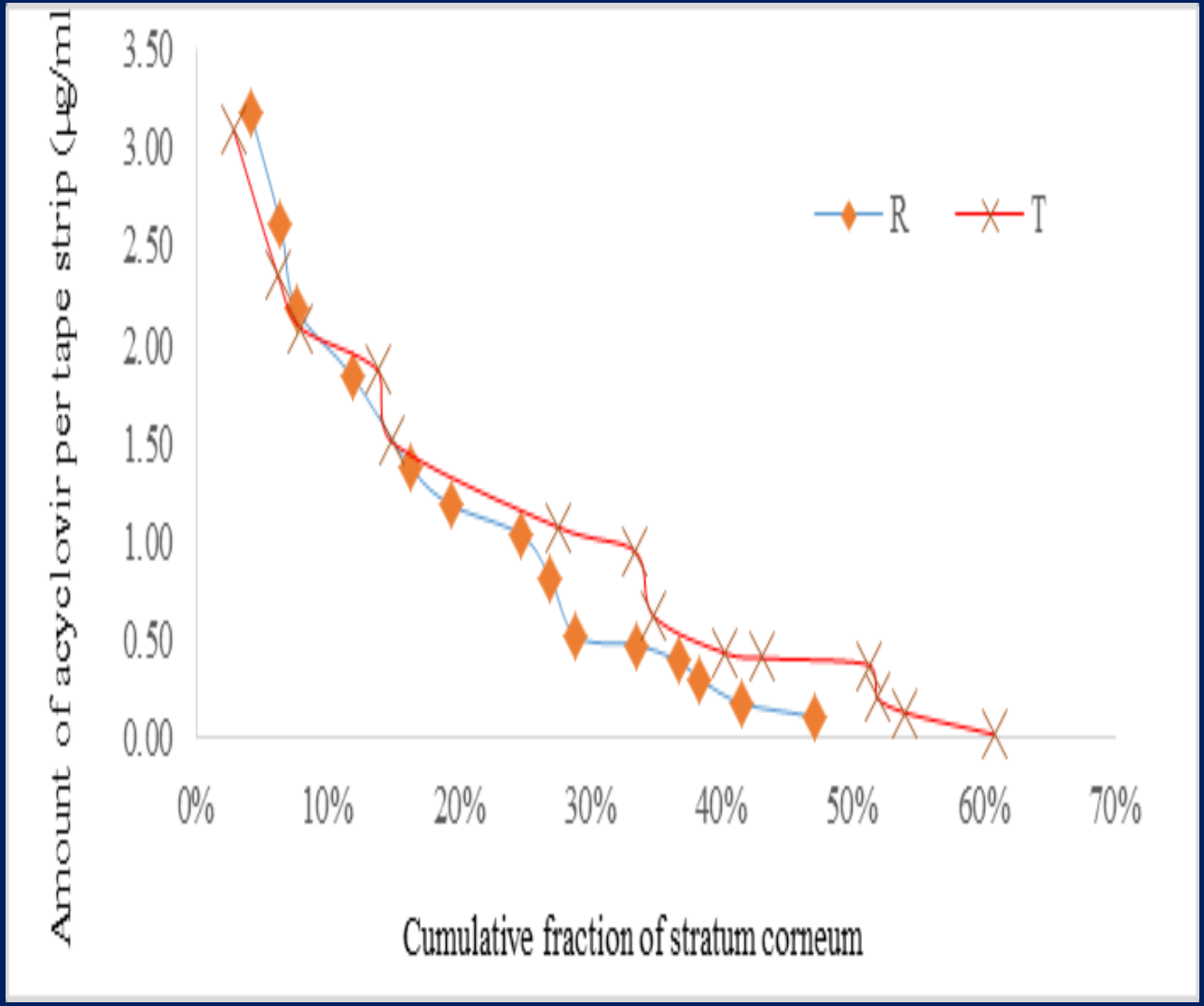
Study population

n = 20 subjects (6 females and 14 males) between the ages of 19 and 30 years (mean 25 years) from Indian descent.

Study design

- 4 application sites and 1 blank site were delineated on the left arm of each subject
- 2 “Test ” (Adco-Acyclovir 5% Cream – Adcock Ingram SA) and 2 “Reference” (Zovirax® 5% Cream – GSK) sites were randomized between individuals


Elbow
A
B
C
D
E
Wrist
LEFT ARM


Amount of acyclowir per tape strip (µg/ml
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0%
10%
20%
30%
40%
50%
60%
70%
R
T
Cumulative fraction of stratum corneum

- 90% confidence interval ($CI_{90\%}$) for the $AUC_{test}/AUC_{reference}$ ratios calculated

- For the untransformed data, the point estimate was calculated by dividing the mean AUC_{test} value by the mean $AUC_{reference}$ value and the $CI_{90\%}$ was determined using Fieller's/Locke's method described in the FDA Guidance for topical corticosteroids

Results for SN_TS2TS data	
Parameter	**Results**
n (number of subjects)	20
$AUC_{test}/AUC_{reference}$	1.03
$CI_{90\%}$	0.93 – 1.18
Bioequivalent? (0.8 – 1.25)	Yes
CV%	25.26%

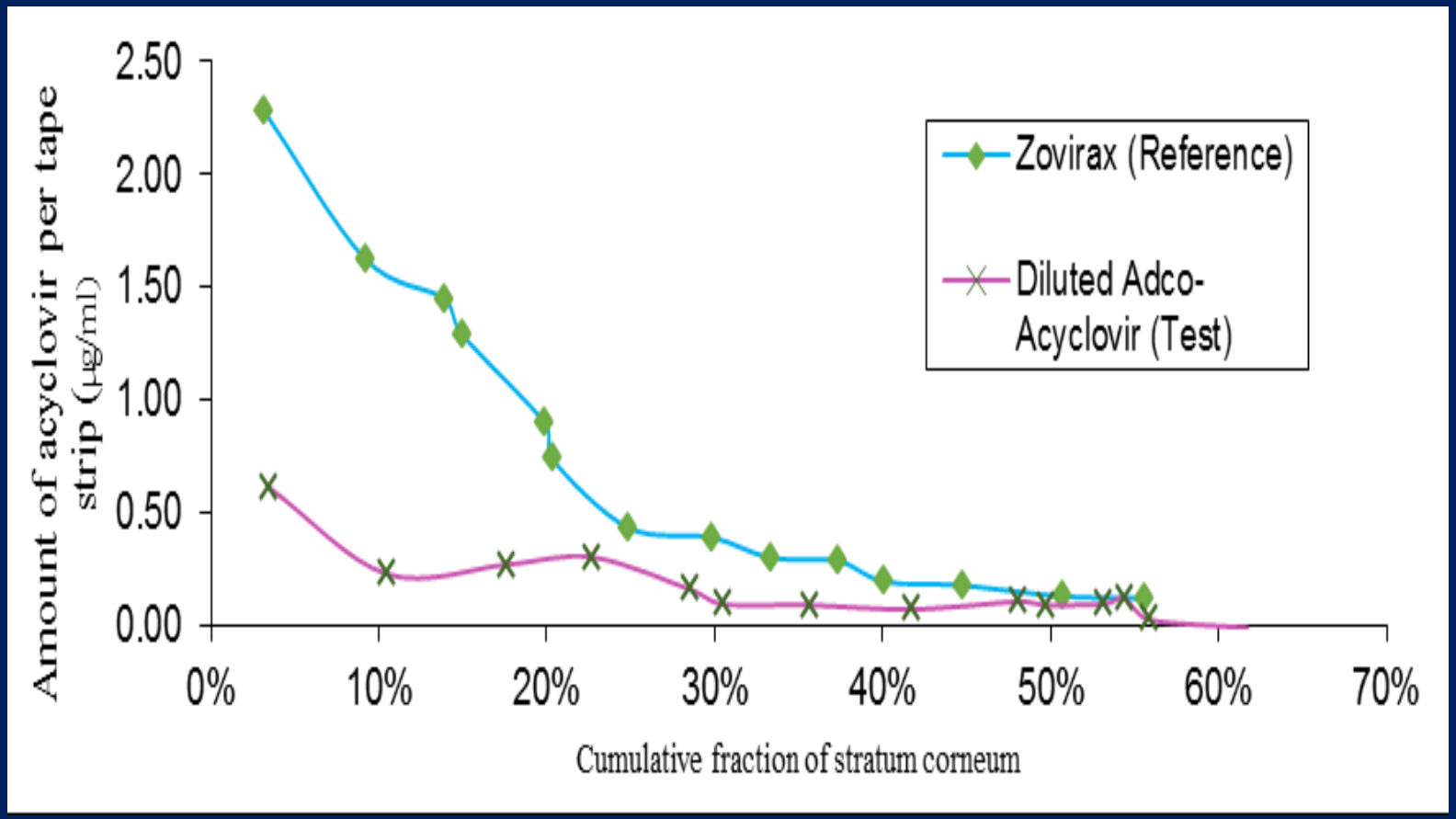
Bioequivalence study of a 30% diluted topical formulations containing acyclovir using TS

Study population

n = 10 subjects (2 females and 8 males) between the ages of 19 and 30 years (mean 25 years) from Indian descent.

Study design

- 4 application sites and 1 blank site were delineated on the left arm of each subject
- 2 “Test ” (Adco-Acyclovir Cream diluted 30% with placebo base) and 2 “Reference” (Zovirax® 5% Cream – GSK) sites were randomized between individuals



Summary of BE results – Diluted T (30%)	
Parameter	**Results**
n (number of subjects)	10
$AUC_{test}/AUC_{reference}$	0.16 – 0.33
Bioequivalent? (0.8 – 1.25)	No

Dermal Microdialysis

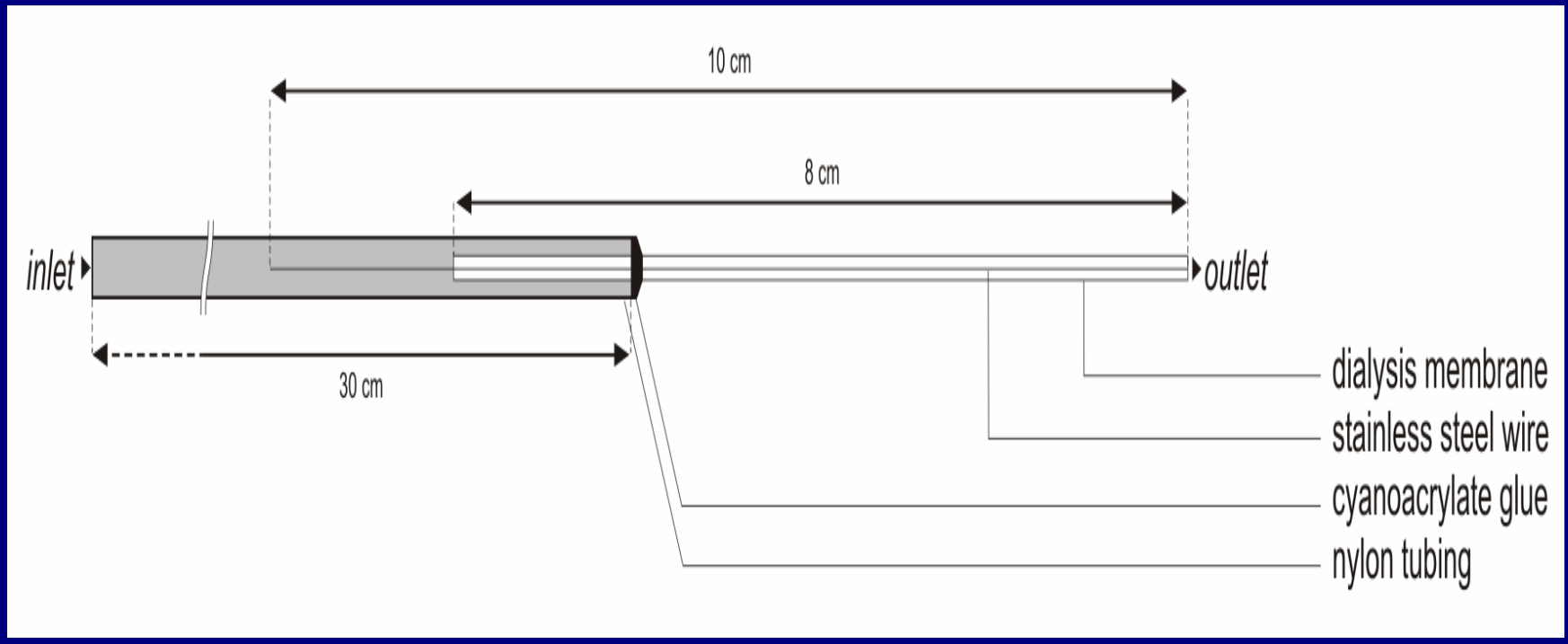
Application of dermal microdialysis (DMD) following the application of a topical formulation to the skin of human subjects in order to evaluate this technique as a tool for the assessment of bioavailability/bioequivalence (BA/BE)

MICRODIALYSIS

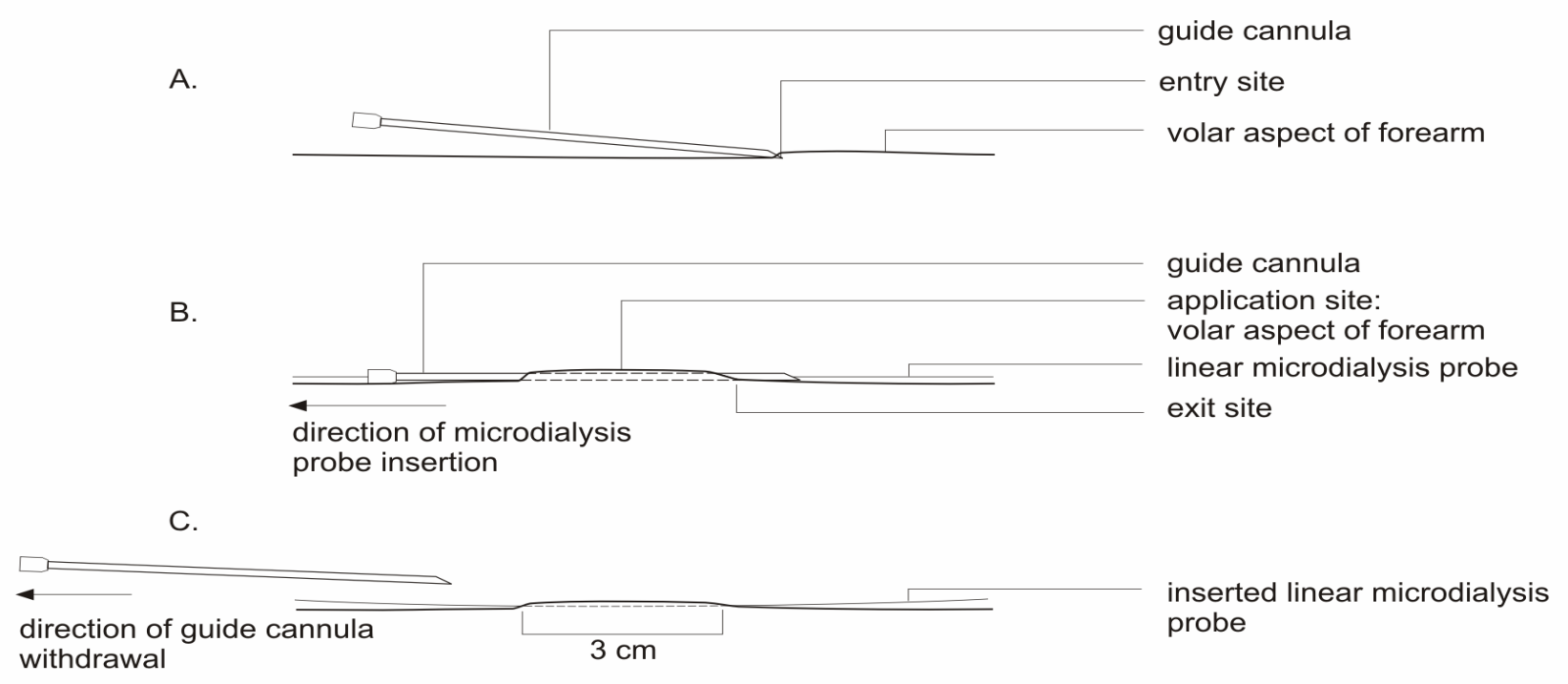
- Microdialysis (MD) - *in vivo* sampling technique to measure endogenous and/or exogenous compounds in extracellular spaces

- Dermal Microdialysis (DMD) is a relatively new application of MD which allows continuous monitoring of endogenous and/or exogenous solutes in the interstitial fluid (ISF) of dermal tissue with minimal tissue trauma

- The technique involves the implantation of a semi-permeable membrane into a specific region of a tissue or fluid-filled space



Linear DMD probe for *in vivo* applications

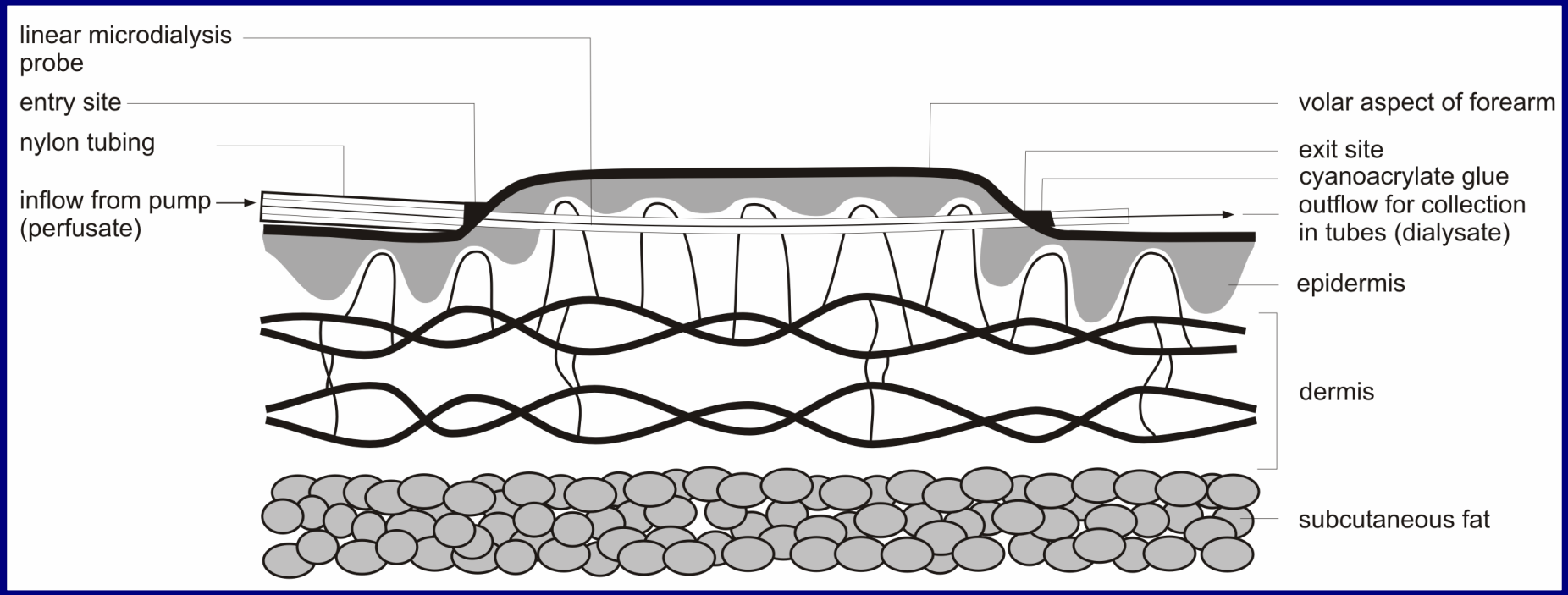


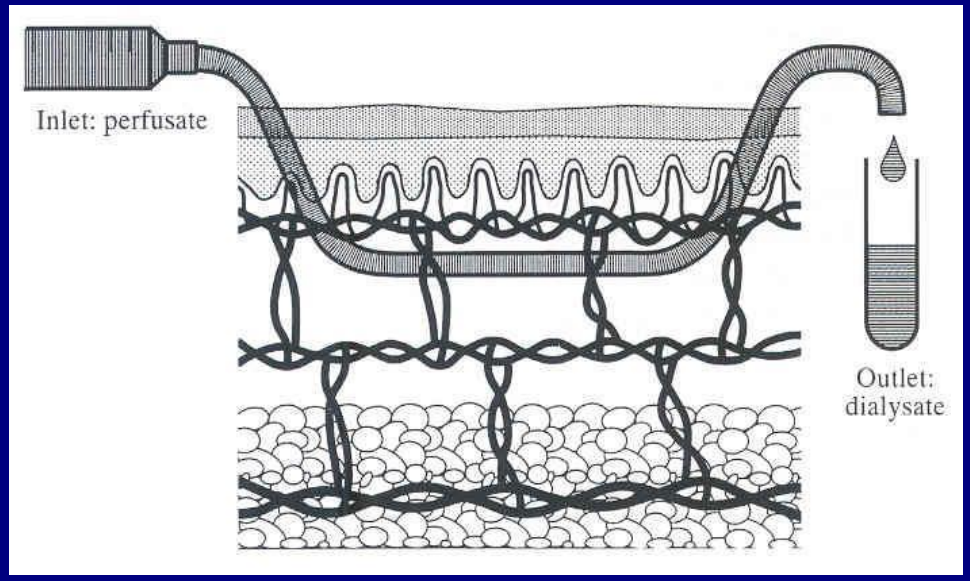
Implantation of linear DMD probes in the skin.

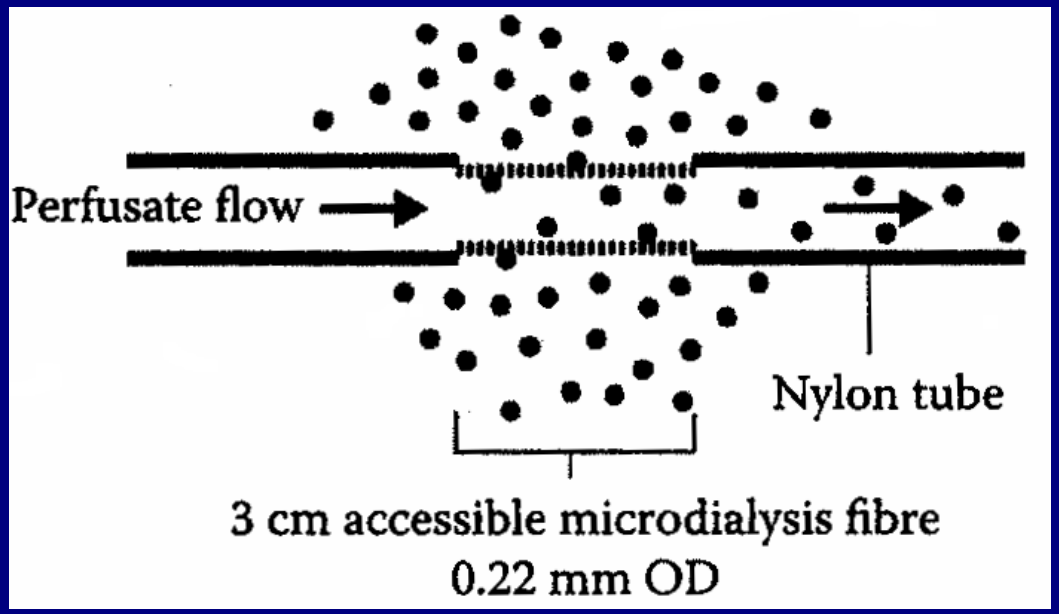
A: Guide cannula insertion at the entry point marked on the skin.

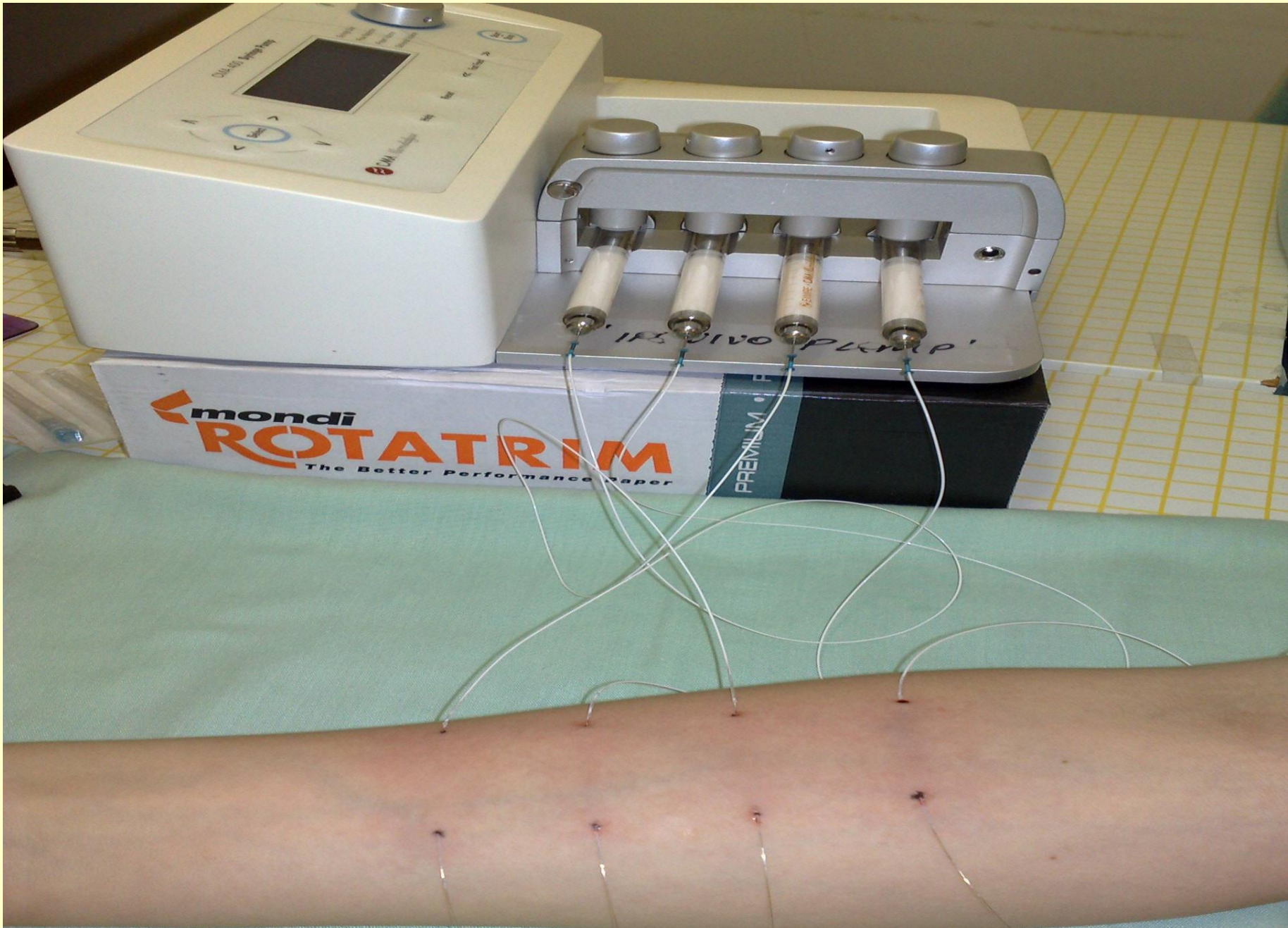
B: Guide cannula pierced through the exit point and MD probes inserted into the guide cannula.

C: Guide cannula withdrawal leaving the MD probe within the dermis


linear microdialysis probe
entry site
nylon tubing
inflow from pump (perfusate)
volar aspect of forearm
exit site
cyanoacrylate glue
outflow for collection in tubes (dialysate)
epidermis
dermis
subcutaneous fat

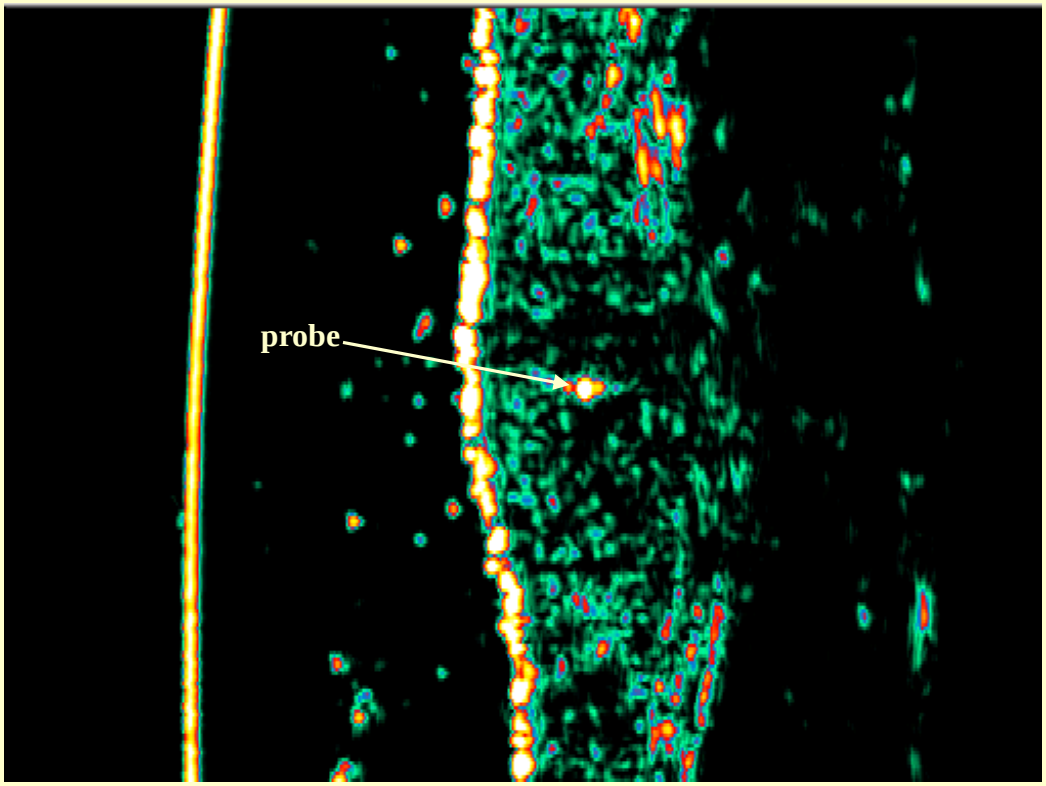

Inlet: perfusate
Outlet:
dialysate


Perfusate flow
Nylon tube
3 cm accessible microdialysis fibre
0.22 mm OD


mondi
ROTATRIM
The Better Performance Paper
PREMIUM

Assessment of Probe Depth

- A probe depth of ~ 0.6 – 1.00 mm
- Ultrasound imaging at 20 MHz



Cross-sectional scan of the skin showing the position of the MD probe

Case Study

- Test formulation
  - Fastum® (ketoprofen 2.5%, m/m) gel formulation
- DMD study design
  - Subjects
    - 18 subjects
      - 9 females and 9 males

Study Design

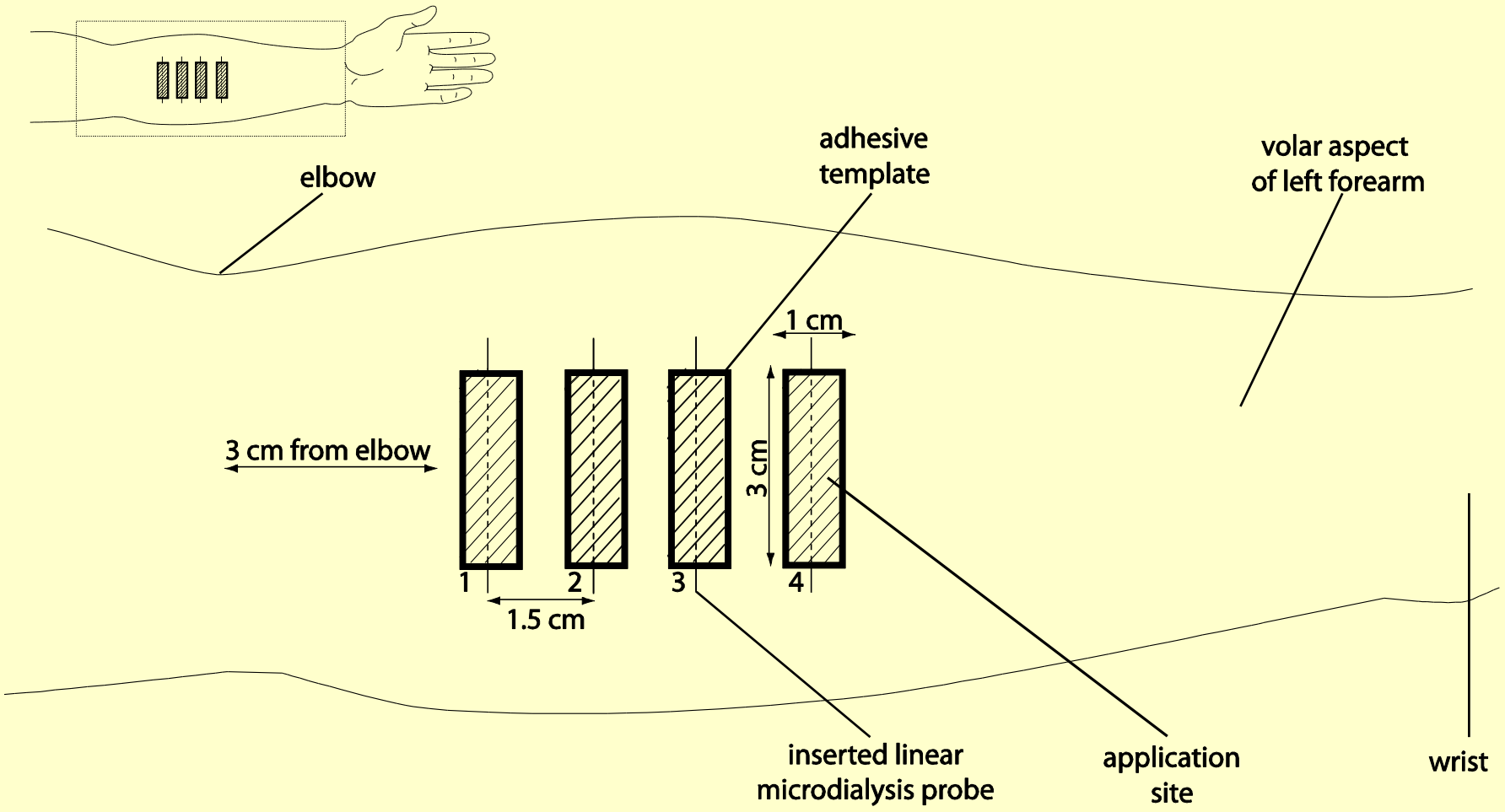
Two sites on each subject were designated as test sites (T) and the other two sites as reference sites (R)

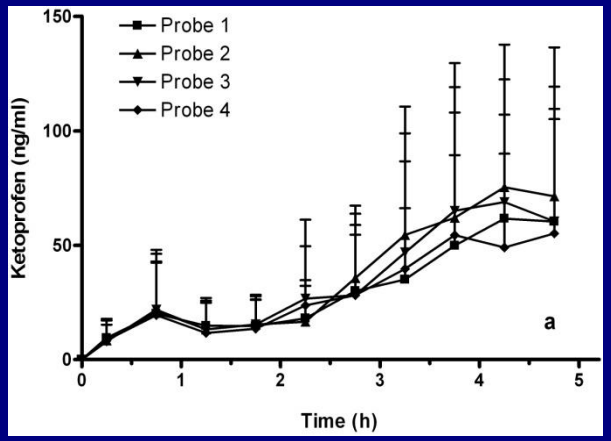
Randomisation sequence:

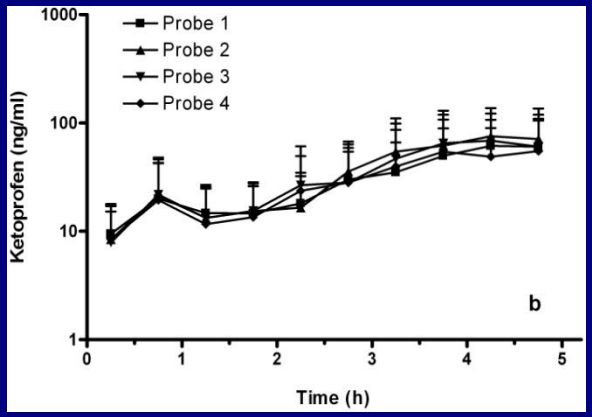
A (TTRR/RRTT), **B** (TRTR/RTRT) and **C** (TRRT/RTTR)
i.e. two pairs of sites for each subject were used in the bioequivalence assessment

Viz:

Sequence A:	Probes 1 and 2 versus 3 and 4
Sequence B:	Probes 1 and 3 versus 2 and 4
Sequence C.	Probes 1 and 4 versus 2 and 3


elbow
adhesive
template
volar aspect
of left forearm
1 cm
3 cm from elbow
3 cm
1
2
3
4
1.5 cm
inserted linear
microdialysis probe
application
site
wrist





Mean dialysate concentration (a) and semi-log (b) time profiles (*n=18*).

Experimental: 4 probe insertions, 4 application sites, 1 probe per site, probes were 1.5 cm apart, probes covered approximately 2 quarters of the volar aspect of the forearm of each volunteer, 18 subjects, Formulation: Fastum® gel (ketoprofen 2.5%, m/m)

Application of dermal microdialysis for the evaluation of bioequivalence of a ketoprofen topical gel.
Tettey-Amlalo RNO, Kanfer I, Skinner MF, Benfeldt E, Verbeeck RK
Eur J Pharm Sci 36:219-225,(2009)

Bioavailability comparison of sequences (*n* = 18).

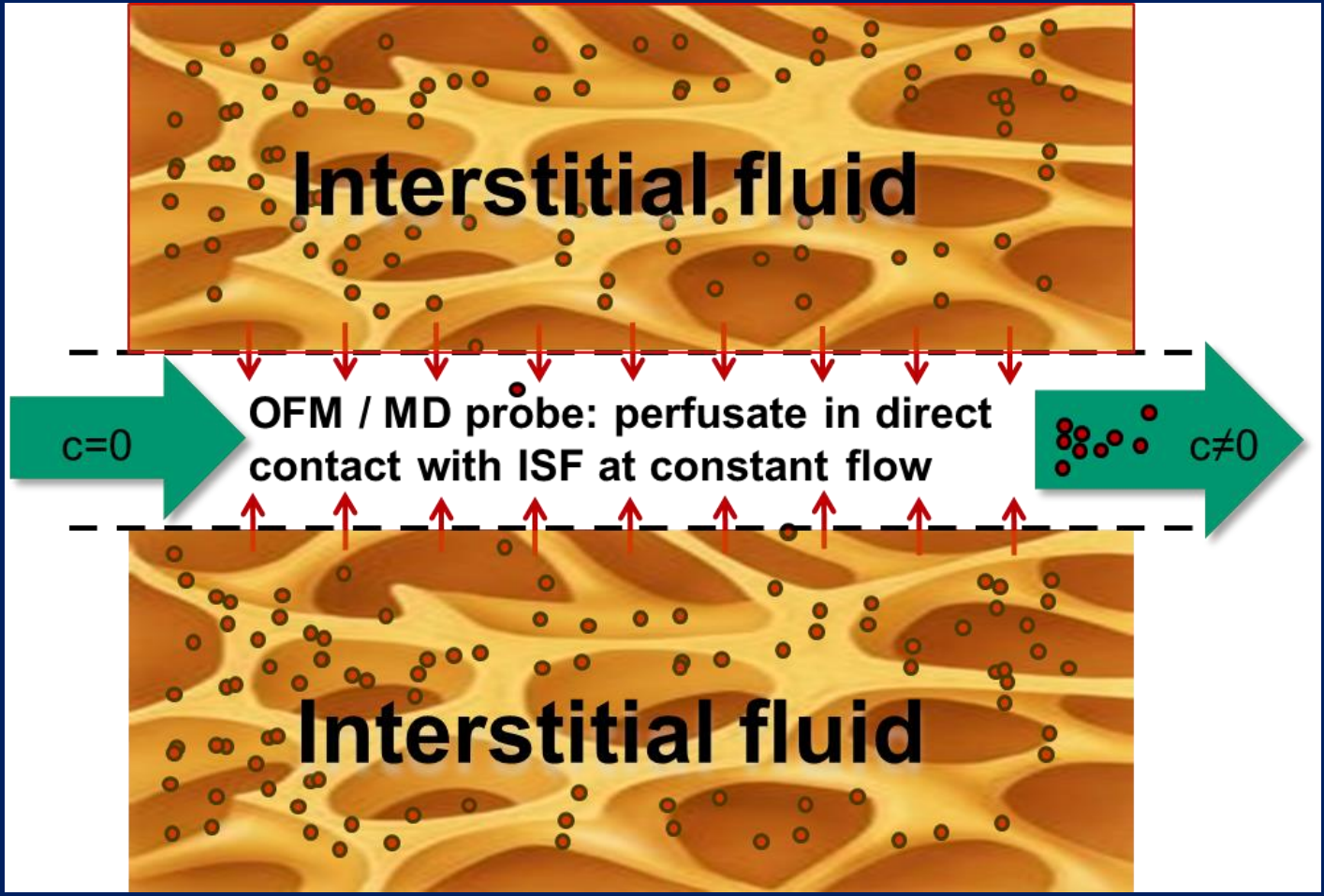
Sequence	PK Parameter (ngh/ml)	%Ratio (S1/S2)	90% CI	Power ANOVA(%)
A	AUC_{0-5}	106.16	(97.39 -115.72)	92.88
B	AUC_{0-5}	99.01	(89.86 - 109.09)	95.95
C	AUC_{0-5}	86.69	(80.37 - 93.50)	53.99

Dermal Open Flow Microperfusion (dOFMD)

- allows continuous sampling of interstitial fluid (ISF) in target tissue
- guarantees direct access to the ISF (whether dermal, adipose or muscle tissue)
- samples interstitial fluid (ISF) directly: no limitations regarding size, protein-binding or lipophilicity of API

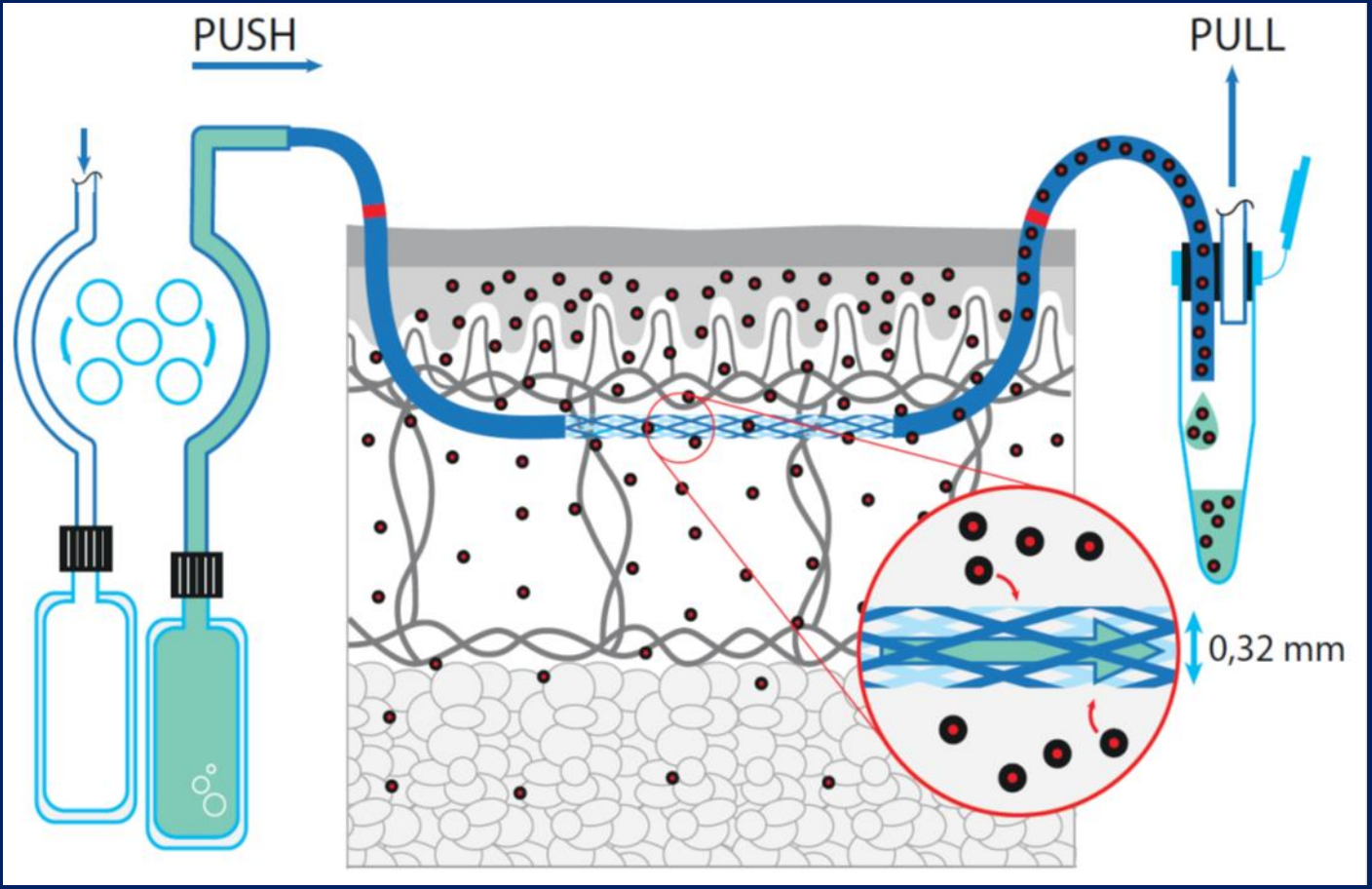
OFM

working principle



OFM

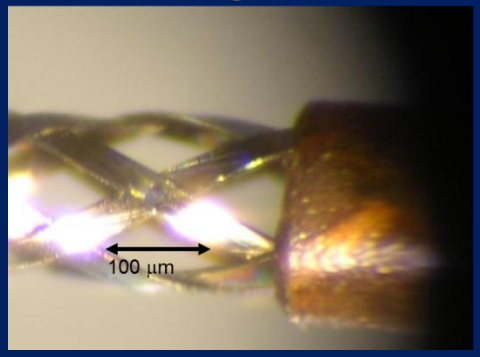
working principle



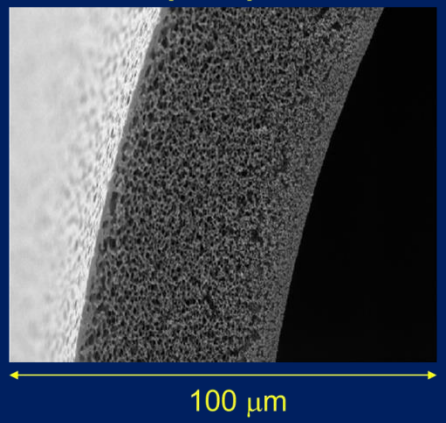
OFM and MD

application range

OFM: 100 µm open exchange areas



MD: membrane, nm-µm pores



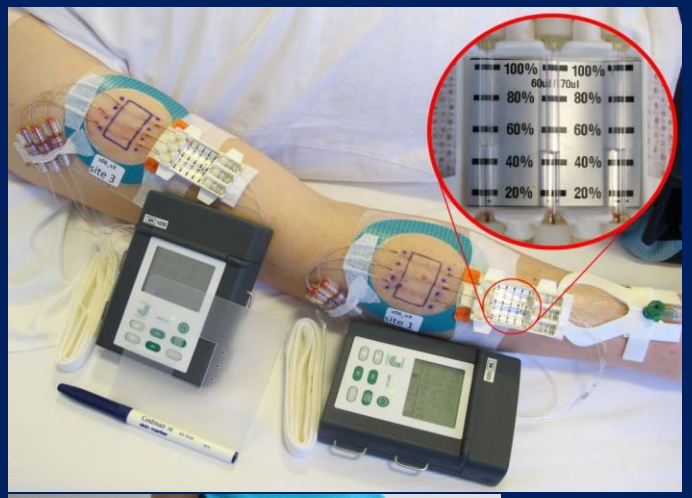
Substance / Drugs …	MD	OFM
… small	YES	YES
… hydrophilic(small)	YES	YES
… larger + large	YES & NO	YES
… lipophilic (super lipophilic)	NO	YES
… protein-bound	NO	YES
… (nano)carrier / cells	NO	YES

MD: PK/PD of small and hydrophilic substances

OFM: PK/PD of ANY substance independent of size and lipophilicity

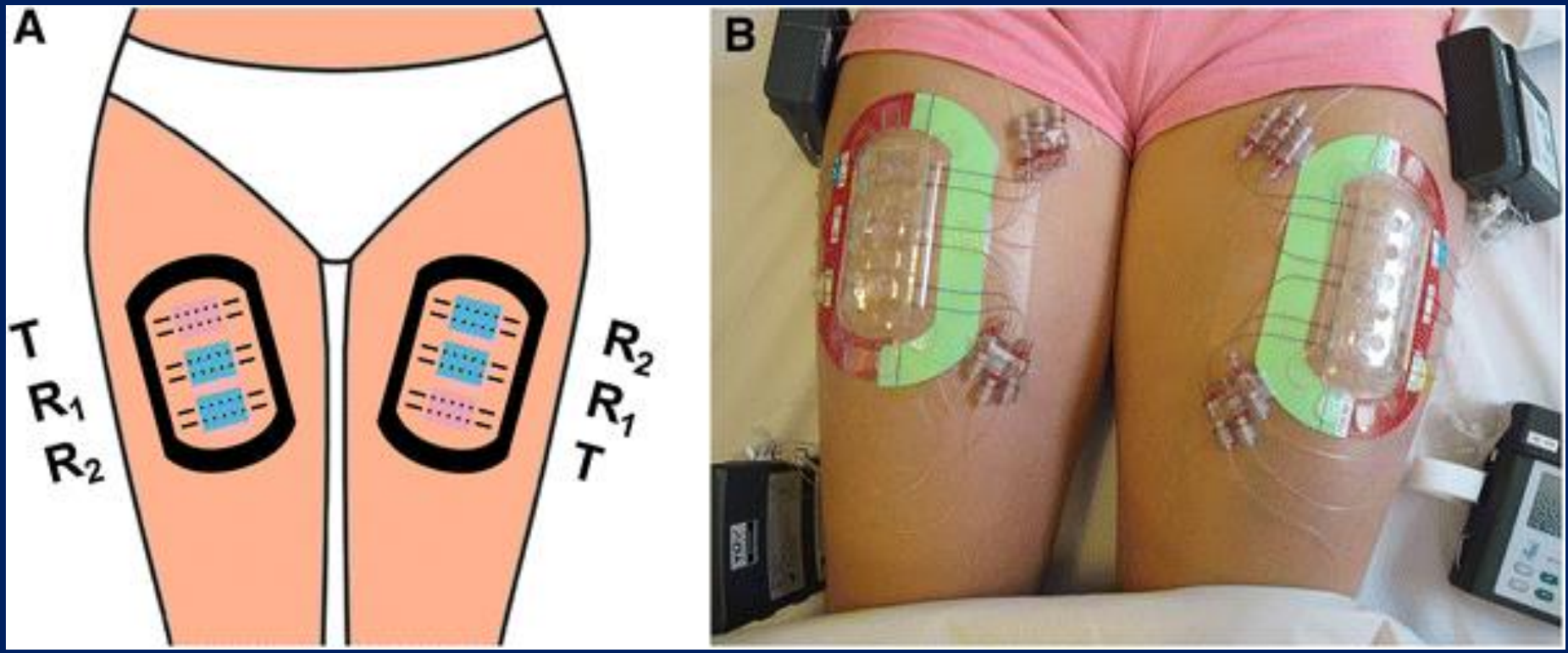
OFM setting

- preclinical use
- clinical use, up to 48h possible
- mobile subjects
- multiple probes
- multiple sites
- multiple tissues (e.g. dermis/cutis; muscle and adipose tissue)

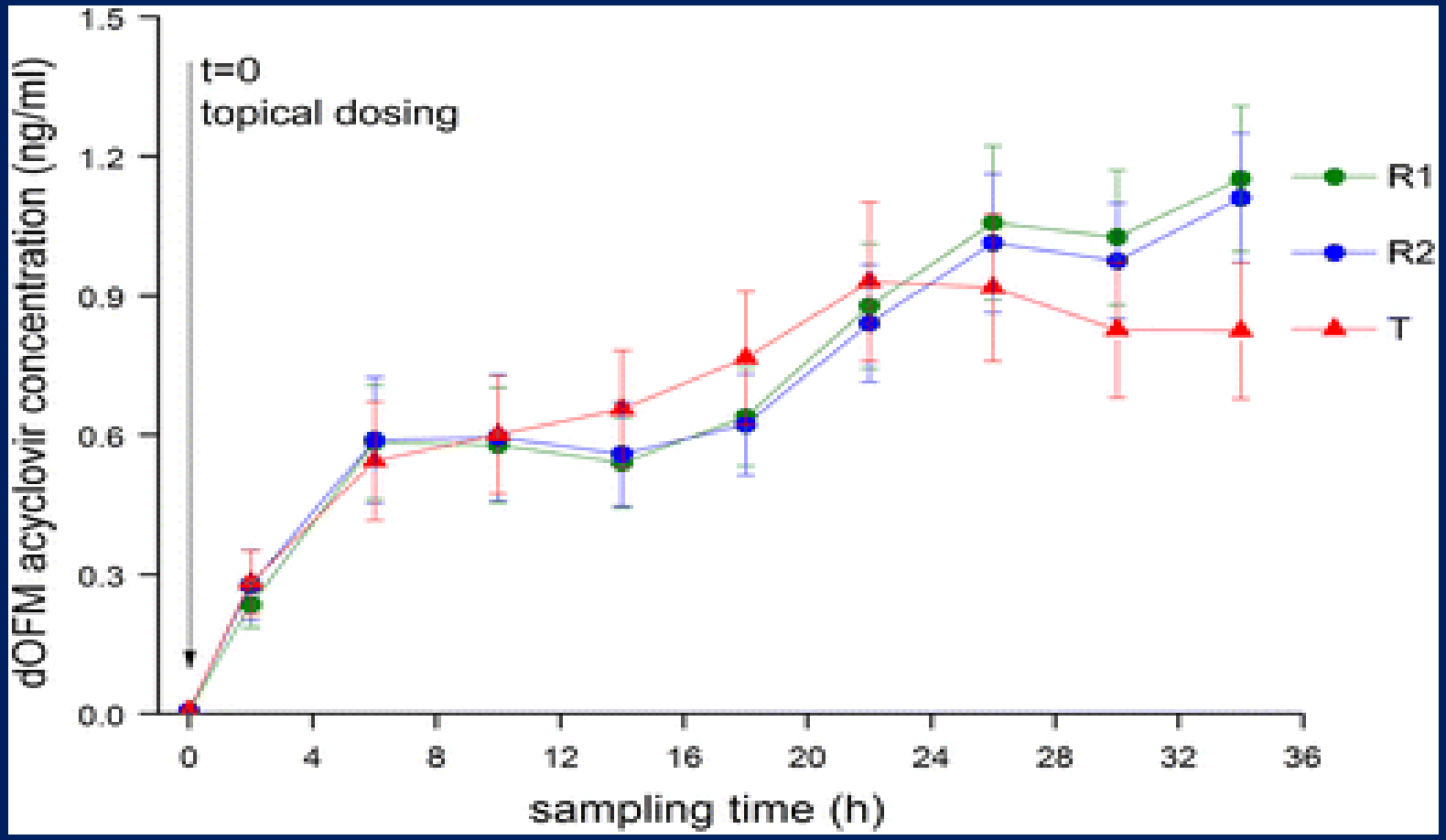


Case Study:

To evaluate whether dOFM is an accurate, sensitive, and reproducible method to characterize the intradermal BA & BE of acyclovir from 5 % acyclovir creams, comparing a reference (*R*) product either to itself or to a different test (*T*) product.



Dermal open flow microperfusion (dOFM) acyclovir concentration profiles for the test product (*T*) site and two reference (R_1 and R_2) products (mean ± standard error of the mean, *n* = 40 test triads in 20 subjects).
Acyclovir was analyzed from one pre-dose sample (spanning −1 to 0 h) and nine pooled post-dose samples (spanning 0–4, 4–8 … 32–36 h).
The post-dose concentrations are plotted at the mid-point of the time intervals (2, 6 … 34 h)



Manfred Bodenlenz, Katrin I. Tiffner, Reingard Raml, Thomas Augustin, Christian Dragatin, Thomas Birngruber, Denise Schimek, Gerd Schwagerle, Thomas R. Pieber,

Sam G. Raney, Isadore Kanfer, Frank Sinner

Open Flow Microperfusion as a Dermal Pharmacokinetic Approach to Evaluate Topical Bioequivalence

Clin Pharmacokinet, *In Press*, 2016

Statistical evaluation comparing PK endpoints using typical BE criteria (n = 40 test triads in 20 subjects)

Comparison	PK endpoint	90 % confidence interval	T/R (point estimate)	Outcome
R_2 vs. R_1	$AUC_{0–36\ h}$	0.86–1.18	1.01	Positive BE result Confirmed R_2 is considered BE to R_1
	C_{max}	0.86–1.21	1.02	
T vs. R_1	$AUC_{0–36\ h}$	0.69–1.05	0.85	Negative BE result Confirmed T is not considered BE to R_1
	C_{max}	0.61–1.02	0.79	

Manfred Bodenlenz, Katrin I. Tiffner, Reingard Raml, Thomas Augustin, Christian Dragatin, Thomas Birngruber, Denise Schimek, Gerd Schwagerle, Thomas R. Pieber, Sam G. Raney, Isadore Kanfer, Frank Sinner

Open Flow Microperfusion as a Dermal Pharmacokinetic Approach to Evaluate Topical Bioequivalence

Clin Pharmacokinet, *In Press*, 2016

PROGNOSIS

- Development and Validation of Surrogate Methods to Assess BE of Topical Products for Local Action

TS

DMD

dOFMD

IVIVC’s

- Acceptance by Regulatory Agencies & Appropriate and Relevant Guidances

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