Research Techniques Made Simple: Drug Delivery Techniques, Part 2: Commonly Used Techniques to Assess Topical Drug Bioavailability

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Assessing the extent of absorption of topical drugs into the various skin layers has been one of the biggest challenges of recent dermatological research. Although skin biopsy samples can be used to directly measure topical drug absorption, biopsies are invasive and not practical for obtaining kinetic data. Common alternative techniques used to assess the bioavailability of topical drugs include in vitro (Franz cell chamber), ex vivo (isolated perfused skin models), and in vivo (vasoconstrictor assay, tape stripping/dermatopharmacokinetics, and microdialysis) techniques. Despite the popularity of these techniques, each technique has its own advantages and disadvantages that limit its use. Consideration of each technique requires that there is a rational linkage to the drug’s clinical endpoint and/or site of action. In this article, we review these in vitro, ex vivo, and in vivo techniques, focusing on the basic concepts and the advantages and disadvantages of each technique.

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INTRODUCTION

Topical drugs are cutaneously applied medications designed to be delivered into the various skin layers. The methodology for assessing the bioavailability of topical drugs requires a multifaceted approach tailored to the drug, disease, and product interface (Shah et al., 2014). Skin biopsy samples can be used to directly measure topical drug absorption. However, biopsies are invasive and are not practical for obtaining kinetic data. Common alternative techniques used to assess the bioavailability of topical drugs include in vitro (Franz cell chamber), ex vivo (isolated perfused skin models), and in vivo (vasoconstrictor assay, tape stripping/dermatopharmacokinetics [DPK], and microdialysis) techniques. Generally, in vitro and ex vivo techniques are used in the early stages of drug development to optimize drug delivery, whereas in vivo techniques are used in the later stages to consider additional local and systemic effects, as well as finalize development.

IN VITRO TECHNIQUES

Franz cell chamber

The most widely used in vitro technique to assess topical drug bioavailability is the Franz cell chamber: a two-compartment system, with a donor compartment and receptor compartment separated by human skin (preferred for most studies), animal skin (typically porcine skin), or an artificial membrane (Figure 1). When skin is used, it can be isolated into epidermis and dermis by enzymatic digestion, heat, chemicals, or dermatox to determine drug absorption parameters for each tissue subsection (Shah et al., 2014). The drug exposure time reflects practical use conditions; in the case of skin membranes, it should not exceed 24 hours to maintain membrane viability.

In the Franz cell, the skin/membrane is mounted in a horizontal position and maintained at 32 ± 1°C. The

SUMMARY AND DEFINITIONS

- There is not one standard technique by which the bioavailability of all topical drugs can be assessed.
- The most commonly used techniques are in vitro (Franz cell chamber), ex vivo (isolated perfused skin models), and in vivo (vasoconstrictor assay, tape stripping/dermatopharmacokinetics, and microdialysis).
- Franz cell chamber: A two-compartment system, separated by excised skin or membrane, that allows calculation of the amount of topical drug that is absorbed through the skin or membrane from the donor compartment to the receptor compartment.
- Isolated perfused skin models: A section of animal skin or an organ, commonly a porcine skin flap, bovine udder, or porcine forelimb, is surgically isolated, then cannulized and perfused with tissue-culture medium and continuously sampled for topically applied drugs.
- Vasoconstrictor assay: A technique that uses the blanching effects of topical corticosteroids as a surrogate marker for their bioavailability.
- Tape stripping/DPK: A technique that measures the amount of topical drug in the stratum corneum using tape strips at sequentially increasing time intervals from the time of application.
- Microdialysis: A technique that involves implantation of a probe with a semipermeable membrane into the dermis or hypodermis, allowing continuous monitoring of topical drugs in the interstitial fluid.

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skin/membrane contains the test drug formulation (e.g., liquid solutions, suspensions, creams, gels, ointments, lotions, pastes, powders, or adhesive patches) (Shah et al., 2014). The donor compartment is on the drug-applied side of the membrane, which can be either open, semi-open, or closed to the atmosphere, depending on which scenario best mimics the real-world application of the drug. The receptor compartment is on the other side of the membrane, which collects the diffused drug/metabolite over time. It contains a fluid that is regularly sampled for drug/metabolite and replaced.

Because the Franz cell is a static system, the receptor fluid needs to be regularly sampled, stirred, and replaced with new fluid. A variant of the Franz cell is the Bronaugh cell, which is a flow-through system with regular perfusion/collection of receptor fluid.

To assess topical bioavailability using the Franz cell, the quantity of the drug/metabolite should be determined in the applicator (spreader, glass rod, loop), donor chamber, surface washings, stratum corneum (SC) (if sampled via tape strips, a technique explained later in this article), skin sample, receptor fluid, and receptor chamber. The drug/metabolite in the receptor fluid, receptor chamber, and skin sample are considered to be the absorbed quantity. As with the Franz cell, if the SC is assumed to contain drug/metabolite available for absorption, the quantity of substance in the SC must be included in the calculation of the total absorbed quantity.

The most convenient method to quantify the drug/metabolite is to radiolabel it. If radiolabeling is not possible, alternative analytical techniques such as liquid chromatography-mass spectrometry (LC-MS), HPLC-mass spectrometry (HPLC-MS), ELISA, or fluorescence can be used (Shah et al., 2014).

In 2010, Salerno et al. used the Franz cell with mixed cellulose ester membranes and full-thickness pig ear skin to show that fluconazole microemulsions containing diethylene glycol monoethyl ether had the greatest skin absorption among various fluconazole formulations.

### EX Vivo Techniques

#### Isolated Perfused Skin Models

Ex vivo techniques involving perfused skin models allow assessment of topical drug absorption with consideration for the effects of microcirculation and metabolism but without the subsequent consequences of systemic absorption (Ehrhardt and Kim, 2008). Commonly used models include the isolated perfused bovine udder, porcine forelimb, or porcine skin flap. The basic foundation of each of these models is the surgical isolation of a section of animal skin or organ with a vascular circulation that can be cannulized, perfused with tissue-culture medium, and continuously sampled for topical drug/metabolite (Figure 2) (Ehrhardt and Kim, 2008; Hobson, 1991).

The topical bioavailability of drugs in the isolated perfused skin models is assessed in a similar manner as it is assessed in the Franz cell. The simplest approach is to quantify the amount of drug/metabolite in the perfusate, surface washings, skin sample, SC (if sampled via tape strips), and core biopsy sample (if performed to determine distribution of drug/metabolite in the skin). The drug/metabolite in the perfusate, skin sample, and core biopsy (if performed) sample is considered to be the absorbed quantity. As with the Franz cell, if the SC is assumed to contain drug/metabolite available for absorption, the quantity of substance in the SC must be included in calculating the total absorbed quantity. This approach to quantifying the absorption of drug is accurate if the venous fluxes at the end of the experiment approach background (Zhai and Maibach, 2004).

Again, the most convenient method to quantify the drug/metabolite is to radiolabel it. If that is not possible, then alternative techniques as mentioned with the Franz cell can be used.

### Figure 2. Schematic of the isolated perfused skin model technique

Perfused skin models involve the surgical isolation of a section of animal skin or organ (most commonly bovine udder, porcine skin flap, or porcine forelimb) with an intact vascular circulation. After the vasculature is cannulized, drug is applied to the surface of the skin/organ, and tissue-culture medium is perfused throughout the model. The perfusate is collected as it exits the skin/organ and analyzed for drug/metabolite.
In 2012, Stahl et al. used both the isolated bovine udder and Franz cell to assess the absorption of topical ibuprofen in various formulations. Although their results indicate general comparability between the isolated bovine udder and Franz cell, they recommend the use of the Franz cell when costs, throughput, and intensity of labor are of concern, and they recommend the use of isolated skin/organs when cell-cell interactions and/or metabolism of the drug in the skin are of concern.

IN VIVO TECHNIQUES
Vasoconstrictor assay

The vasoconstrictor assay is a technique that uses the blanching effects of topical corticosteroids as a pharmacodynamic endpoint to determine their bioavailability (Figure 3). Skin blanching with topical corticosteroid application is due to local vasoconstriction and can be measured by chromametry, digital image analysis, or visual inspection.

Chromametry involves quantifying the reflection of white light with the following three parameters: red-green, yellow-blue, and light-dark (Bronaugh and Maibach, 2002). Similarly, digital image analysis involves the capture of a 0.5-cm² skin site image at 300 dots per inch that is subsequently analyzed using the same three parameters (Bronaugh and Maibach, 2002). Unlike the other two methods, visual inspection involves no technical equipment; it requires only a naked eye examination and the 0–4 score scale introduced by Barry and Woodford (absent = 0, faint = 1, faint-moderate = 2, moderate-strong = 3, and strong-intense blanching = 4) (Barry and Woodford, 1975). Currently, chromametry is preferred by regulatory agencies, because of its reliability and reproducibility. Nevertheless, visual inspection is still considered by some to be a valid method of assessment with sufficient training and experience. As for digital image analysis, although it provides a very attractive alternative to chromametry and visual inspection, further research is needed to determine its practical applicability (Kanfer, 2010; Shah et al., 2014).

In 2008 Au et al. used both visual inspection and chromametry to assess the skin blanching effects of topical clobetasol propionate. Their results indicate that the visual and chromameter assessment methods are comparable to each other and equally applicable for evaluating the vasoconstrictor assay.

Tape stripping/DPK

Tape stripping, or DPK, is a technique that allows measurement of the amount of topical drug in the SC of human skin. The DPK method assumes that (i) the SC is the rate-limiting barrier to topical absorption and (ii) drug levels in the SC are directly related to drug levels in the underlying epidermis (Herkenne et al., 2008; Herkenne et al., 2007). To assess the absorption of topical drugs using tape stripping, the drug is applied at multiple sites, and the SC is collected via tape strip from each site just after removing the applied drug at sequentially increasing time intervals from the time of application. Approaches used to quantify the amount of SC and drug/metabolite removed include weighing the tape strips before and after stripping, various assays of proteins in corneocytes, radiolabeling, HPLC, and/or optical spectroscopy (Herkenne et al., 2008; Shah et al., 2014). Regardless of whether the weighing method is used for quantification, it is still required for standardization of various tape strip and SC variables (Herkenne et al., 2007).

After the drug/metabolite is quantified on each tape strip and total epidermal water loss measurements are made using Fick’s first law, it is possible to graph the concentration of the drug/metabolite as a function of its relative depth into the SC, yielding the following parameters after curve fitting to an appropriate solution of Fick’s second law: $K$ (the SC-vehicle partition coefficient of the drug/metabolite) and $D/L^2$ (a first-order rate constant for drug/metabolite transport through the SC). Once $K$ and $D/L^2$ are determined, they can be used to yield the $C_{max}$ (the maximum concentration of drug/metabolite in the skin), $T_{max}$ (the time at which the maximum concentration is achieved), and area under the curve (AUC, in a plot of drug/metabolite concentration as a function of time, i.e., the total amount of drug/metabolite in the SC at the end of the application period) (Figure 5). Additionally, with

Figure 3. Vasoconstrictor assay on the volar forearm. After a topical corticosteroid is applied to the skin, it causes skin blanching due to local vasoconstriction. This effect is quantified using chromametry, digital image analysis, or visual inspection, and it is used as a pharmacodynamic endpoint to assess bioavailability. Adapted from Marshall et al. (2010) with permission from the American Chemical Society.

Figure 4. Schematic of the tape stripping/dermatopharmacokinetics technique. Tape stripping measures the amount of topical drug/metabolite in the stratum corneum of human skin. Drug is first applied to multiple sites and allowed to absorb into the skin for a certain amount of time. Then, the SC is collected via tape strip from each site after removing the applied drug, at sequentially increasing time intervals from the time of application.
knowledge of $D/L^2$, the classic lag time for diffusion across the SC can be determined, as can the time necessary to reach steady state transport. If these parameters do not change over time, which is not always the case, the complete absorption profile of the drug can be derived from a short-duration experiment.

In 2007, Herkenne et al. used the tape stripping technique to derive $K$ and $D/L^2$ for topical ibuprofen, yielding its absorption profile up to steady state. Their technique builds upon and addresses many of the concerns of the original 1998 United States Food and Drug Administration draft guidance for tape stripping, which was withdrawn in 2002 because of unreliability. Further support for the use of tape stripping was provided by Benfeldt et al. in 2007, when they found low variability and a similar rank-order correlation with tape stripping as with microdialysis.

**Microdialysis**

Microdialysis is a technique that allows real-time, continuous monitoring of the extracellular concentration of drug/metabolite in the dermis and hypodermis. Most commonly, a linear probe with a semipermeable membrane is implanted into the dermis or hypodermis with the help of a guide needle. The probe functions as an artificial vessel with an inlet and outlet tube that is perfused with a physiological solution, which equilibrates with the interstitial fluid, allowing passive diffusion of substances smaller than the cut-off value of the membrane (Figure 6) (Ehrhardt and Kim, 2008).

Insertion of the probe and guide needle is a minimally invasive procedure that requires small skin punctures and provokes minor tissue trauma that generally resolves to baseline after 60–90 minutes. The perfusate is typically isotonic saline or Ringer’s lactate solution and is pumped at a very slow flow rate (1–5 µl/min). If the drug is lipophilic, solvents may have to be added to the solution to allow better solubility. The perfusate is typically analyzed using HPLC-MS, but it can be analyzed using alternate techniques, such as ultra-performance LC-MS or other specific biosensors. As was the case with tape stripping, the absorption profile of the drug (the $C_{\text{max}}$, $T_{\text{max}}$, absorption constant, AUC, and lag time) can be determined from the plot of free drug/metabolite concentration in the dermis or hypodermis as a function of time (Figure 5) (Ehrhardt and Kim, 2008; Herkenne et al., 2008).
A variant of microdialysis is open-flow microperfusion, which uses probes with macroscopic openings rather than porous membranes. Compared with microdialysis, open-flow microperfusion allows open exchange with the interstitial fluid, with fewer limitations due to molecular size, drug protein-binding, and drug lipophilicity (Ehrhardt and Kim, 2008).

In 2009, Tettey-Amlalo et al. used the dermal microdialysis technique to derive the AUC for topical ketoprofen, yielding its absorption profile from 0 to 5 hours. Interestingly, they also applied the same ketoprofen gel at reference and test sites on human subjects to validate the use of microdialysis as a tool to compare different drug formulations.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>In vitro</td>
<td></td>
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<tr>
<td>Franz/Bronaugh Cell Chamber</td>
<td>• Useful in devising delivery vehicles</td>
<td>• Limited when studying lipophilic compounds</td>
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<td></td>
<td>• Takes advantage of similarities between animal (typically porcine) and human skin</td>
<td>• Inherent animal and human skin variability</td>
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<td></td>
<td>• Inexpensive</td>
<td>• Difficulty of obtaining human skin</td>
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<td></td>
<td>• Quick results</td>
<td>• Does not allow consideration of in vivo skin processes</td>
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<td></td>
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<td>• Although attempts have been made to create a membrane modeling diseased human skin, there is currently no accepted way to accurately do so</td>
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<td>Ex vivo</td>
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<tr>
<td>Perfused skin models</td>
<td>• Allows consideration of the effects of microcirculation and metabolism without systemic involvement</td>
<td>• Requires labor-intensive preparation (porcine skin flap)</td>
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<tr>
<td>• Isolated bovine udder</td>
<td>• Does not require labor-intensive preparation (bovine udder)</td>
<td>• Inherent animal and human skin variability</td>
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<tr>
<td>• Isolated porcine skin flap</td>
<td>• Takes advantage of similarities between animal and human skin</td>
<td>• Although bovine skin has successfully been altered in attempts to model diseased human skin, there is currently no accepted way to accurately perform the technique</td>
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<tr>
<td>• Isolated porcine forelimb</td>
<td>• Live skin processes somewhat considered</td>
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<tr>
<td>In vivo</td>
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<tr>
<td>Vasoconstrictor assay</td>
<td>• Can indicate drug efficacy</td>
<td>• High subject-to-subject variability</td>
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<td>• Live skin processes somewhat considered</td>
<td>• Limited to drugs that induce local vasoconstriction (corticosteroids)</td>
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<td></td>
<td>• Applicable for diseased skin</td>
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<tr>
<td>Tape stripping/dermatopharmacokinetics</td>
<td>• Inexpensive</td>
<td>• Less useful when the target of the drug is the dermis</td>
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<td>• Useful in measuring efficacy of sunscreens, antifungal agents, and antiseptics</td>
<td>• Time consuming</td>
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<td>• Live skin processes somewhat considered</td>
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<td>• Applicable for diseased skin</td>
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<tr>
<td>Microdialysis</td>
<td>• Provides continuous, real-time monitoring</td>
<td>• Slightly invasive</td>
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<td></td>
<td>• No fluid extraction from tissue</td>
<td>• Difficult to reproduce consistent depth of probe</td>
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<td>• Fairly reproducible</td>
<td>• Limited when studying lipophilic compounds</td>
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<tr>
<td></td>
<td>• Live skin processes somewhat considered</td>
<td>• Very low concentrations are difficult to measure</td>
</tr>
<tr>
<td></td>
<td>• Has been shown to be applicable for diseased skin and may be superior to tape stripping for diseased skin</td>
<td>• Limited use with slowly penetrating substances</td>
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<td>Open flow microperfusion</td>
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<td>• Fairly reproducible</td>
<td>• Limited use with slowly penetrating substances</td>
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<td></td>
<td>• Live skin processes somewhat considered</td>
<td>• Samples require pretreatment before analysis</td>
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<tr>
<td></td>
<td>• Fewer limitations than microdialysis with respect to molecular size, drug protein-binding, and drug lipophilicity</td>
<td>• Measures only total drug (including protein bound)</td>
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<tr>
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MULTIPLE CHOICE QUESTIONS

1. Which of the following types of skin and/or membrane is typically preferred in the Franz/Bronaugh cell chamber?
   A. Human skin
   B. Animal skin
   C. Artificial membrane
   D. All of the above are equally preferred

2. What is a unique advantage of isolated perfused skin models?
   A. Allows assessment of the bioavailability of any topical drug
   B. Takes into consideration the effects of microcirculation and metabolism, without systemic involvement
   C. Allows the blanching effects of topical drugs to be used as a surrogate marker for bioavailability
   D. Uses human skin without requiring live human subjects

3. What causes the unique blanching effects of topical corticosteroids?
   A. Neurotoxins
   B. Lipophilicity
   C. Local vasoconstriction
   D. Tachyphylaxis

4. Why are tape strips always weighed before and after application when they are used to determine the bioavailability of topical drugs?
   A. To increase the absorption of the topical drug
   B. To use the data in other experiments
   C. To standardize various tape strip and stratum corneum variables
   D. No particular reason

5. If the test drug is lipophilic, what adjustment can be made to the microdialysis technique to improve outcomes?
   A. Add solvents to the solution to allow better solubility
   B. Increase the flow rate to 100–200 μl/min
   C. Repeat the study multiple times
   D. No adjustments can be made

CONCLUSION

There is not one standard technique by which the bioavailability of all topical drugs can be assessed. Each of the techniques mentioned in this article has its own advantages and disadvantages (Table 1). Consideration of using each technique requires that there is a rational linkage to the drug’s clinical endpoint and/or site of action. Based on research between pharmaceutical scientists and dermatologists, it has been suggested that multiple techniques be used in complement to take advantage of each technique’s assets (Yacobi et al., 2014). In the future, there is a need for greater attention to quality, creativity, and variety to identify new techniques that are based on the underlying science and adapted to the drug and disease process being studied.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES


