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MINI-REVIEW

Optimizing *In Vitro* Skin Permeation Studies to Obtain Meaningful Data in Topical and Transdermal Drug Delivery

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Abstract

Drug delivery through the skin provides several advantages over other administration routes, including the avoidance of first-pass metabolism and gastrointestinal side effects, prolonged drug release, and significant improvement in patient compliance. It is imperative to study the *in vitro* behavior of drugs and formulations before proceeding to *in vivo* evaluations. As the ethical guidelines for scientific research evolve, there is an increasing emphasis on adopting alternative methods to reduce animal use. An *in vitro* permeation study (IVPT) estimates the rate and extent of drug permeation from a topical or transdermal delivery, determining its availability at the skin layers or into the systemic circulation. Vertical Franz diffusion cells are commonly employed for IVPT studies to evaluate the permeation of drugs across skin or other biorelevant membranes. This comprehensive review provides a clear understanding of the importance of optimizing *in vitro* experimental conditions to obtain reliable and reproducible data. We discuss various *in vitro* skin models, including excised human and animal skins, human skin equivalents (HSEs), synthetic membranes, and 3D-printed skin models. Additionally, a broad overview of setting up *in vitro* diffusion cells is provided. Emphasis is given on donor phase design, receptor medium selection, the importance of solubility and stability studies, sampling techniques, and analysis methods. Meticulous design and optimization of *in vitro* permeation experiments are crucial for generating reproducible data, which are essential for predicting the dermatokinetics of drugs and formulations.

Keywords 3-D printing · human skin · *in-vitro* · permeation · synthetic membrane

Introduction

Transdermal drug delivery (TDD) offers many advantages over oral administration such as the avoidance of first-pass metabolism and gastrointestinal disturbances and a reduced dosage frequency [1, 2]. Compared to the parenteral route, TDD is non-invasive, it can be administered easily without the need for a healthcare professional, and in the event of any side effects, or if a change in therapy is required, then the transdermal system may be easily removed [3, 4]. Drug release from transdermal therapeutic systems occurs in a controlled or sustained manner over a prolonged period, thereby avoiding the fluctuations in serum drug concentration as seen with oral and bolus intravenous administration [5]. The efficacy of any pharmaceutical formulation is ultimately to be proven in a clinical setting. However, before proceeding to *in vivo* experiments, *in vitro* efficacy testing is usually employed to predict the permeation behaviour of drugs and formulations. *In vitro* skin permeation testing (IVPT) are vital in evaluating the performance of TDD systems and they require careful optimisation of several parameters to obtain robust data. The selection of suitable skin models, diffusion cell design, receptor phase compositions, sampling and analysis play vital roles in obtaining reliable data for predicting rational *in vitro- in vivo* correlations (IVIVC) [6].

Drug Diffusion Mechanisms Across the Skin Layers

The permeation of drugs across the skin can be influenced by the hydration level of the skin; deeper layers of the skin have a higher water content which decreases significantly

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nearer the surface of the skin, particularly in the stratum corneum (SC) where the high lipid content selectively promotes the permeation of lipophilic drugs [7]. There are two main pathways involved in the permeation of drugs across the skin: the transappendageal pathway and the transepidermal pathway [8]. The transappendageal pathway is through the natural openings in the skin such as hair follicles and sweat ducts, and this channel is considered largely hydrophilic. The limitation of this route is that these openings constitute only 0.1% of the total skin surface area and there will be sweat and sebum flowing in the opposite direction. The transepidermal pathway is further subdivided into two: the intercellular pathway, which is a convoluted route through the intercellular lipid spaces; the other is the transcellular pathway, where drug molecules diffuse directly through both corneocytes and intercellular lipid lamellae [9] (Fig. 1).

Diffusion through the hydrophilic and lipophilic layers of the skin is extremely difficult for most drugs and, for this reason, the intercellular pathway may be the preferred route of permeation. However, permeation largely depends on the physicochemical properties of the permeating molecule and there is often permeation through the skin via a combination of all the three pathways mentioned above [10]. Therefore, an ideal transdermal drug candidate must possess balanced lipophilic and hydrophilic characteristics. Highly lipophilic drugs penetrate the SC faster, but their permeation through the relatively hydrophilic dermal layers is limited, conversely, highly hydrophilic drugs pose difficulty to cross the SC [11].

Skin Models for *In Vitro* Permeation Studies

In vitro permeation experiments using suitable skin models can be a better alternative to *in vivo* animal studies in cases where ethical issues are of major concern. Moreover, *in vitro* experiments are more cost-effective than studies using live subjects. There are several skin models that can be used for *in vitro* studies including human or cadaver skin, animal skin, human skin equivalents and other synthetic membranes [12].

Human and Animal Skin Models

Human skin is commonly employed for *in vitro* experiments due to its better correlation with *in vivo* data than other skin models. The skin can be collected from patients undergoing plastic surgery or may be cadaveric [13]. Human research ethics approval must be obtained for all studies involving human tissue. Tissue donors should be provided with an information sheet that clearly explains the purpose of the research and the disposal process for their donated skin. Skin samples must only be collected after obtaining their informed consent. The subcutaneous fat can be removed by blunt dissection, and the skin can then be cut into pieces according to the dimensions of the diffusion cell and stored at − 20°C. Reports suggest that excised human skin can provide comparable results to *in vivo* models since freshly excised can sustain its metabolic activity up to several days [14]. When drug permeation through the epidermal layer is

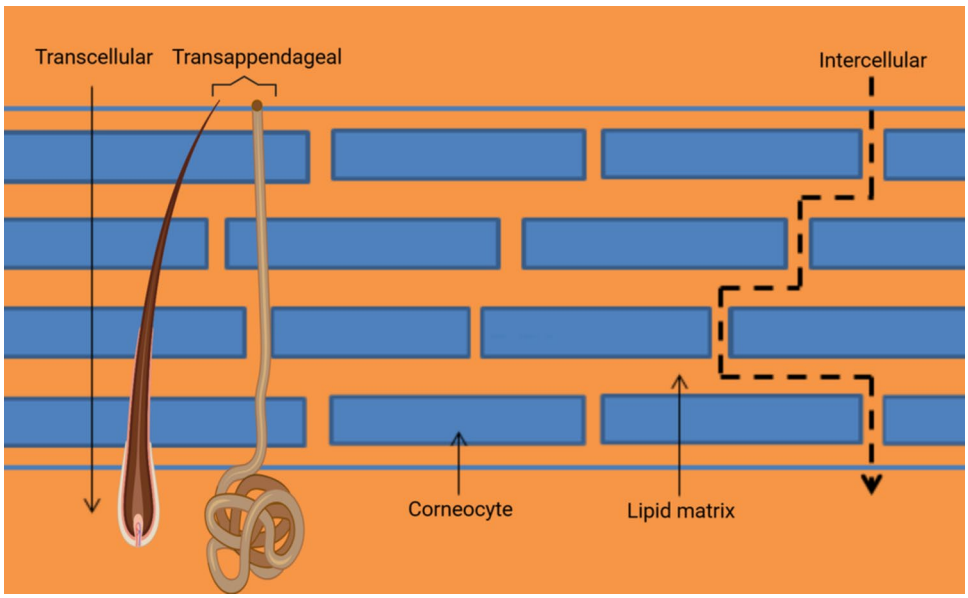

Fig. 1 Transdermal permeation of drugs showing transcellular, transappendageal and intercellular pathways

to be evaluated, the epidermis can be separated from full-thickness human skin by immersing it in deionized water at 60°C for about one minute. After brief immersion, the epidermis can be carefully peeled away from the dermis using surgical forceps [15].

As human skin is not always readily accessible, researchers often use excised animal skin for *in vitro* experiments. Commonly used models include skin from pigs, rats, snakes and mice [16]. Among these models, porcine ear skin is probably most similar to human skin with a comparable SC thickness [17]. The viability of excised animal skin can also be maintained if it is freshly excised. However, permeation data obtained using excised animal skin cannot always be extrapolated to humans.

Human Skin Equivalents (HSEs)

HSEs are composed of tissue-engineered skin cells along with extracellular matrix such as collagen. These HSEs are generally used for skin transplants and also for permeation studies. HSEs can be designed to resemble different types of skin membrane, e.g. epidermis, dermis or full-thickness skin. An ideal HSE should possess a comparable morphology to that of human skin with similar structures and lipid contents [18]. Researchers develop HSEs similar to real skin using primary cells such as stem cells, keratinocytes and fibroblasts along with extracellular components. They are also gaining more attention from regulators as an alternative to animal models. HSE can be classified into living skin equivalents (LSEs), which are models of the entire human skin, and the simple epidermis model which is the reconstructed human epidermis (RHE) (Fig. 2). In this model, a single layer of human keratinocytes grown on a polycarbonate filter at the air–liquid interface. It resembles the human epidermis layer and is generally beneficial in studying permeation and skin irritation. On the other hand, LSEs are more complex models made out of human skin cells from several layers, such as the dermis, epidermis, and occasionally the subcutaneous tissue. They are used for investigating various skin diseases and wound healing as they closely resemble to the structure and function of human skin than the RHE model [19].

Synthetic Membranes

The use of *ex vivo* skin models is the primary source that has been utilised over the past and continues to be the primary method for the optimisation of topical and transdermal drug formulations. However, due to the increasing concern about animal sacrifice and human skin availability, alternative *in vitro* skin models are becoming increasingly popular for permeation experiments [20]. Although synthetic membranes

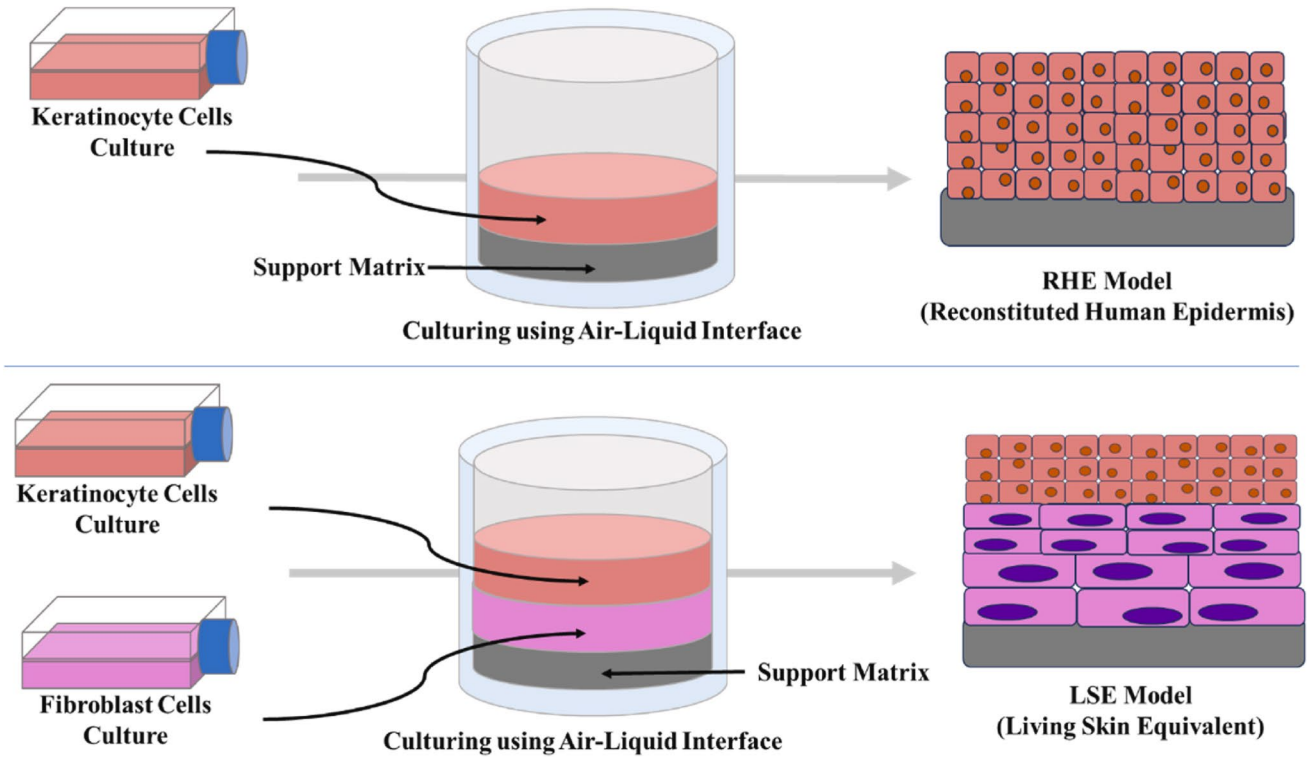

Fig. 2 Schematic representation of Artificial Cultured Human Skin Equivalent. Adapted with permission from Suthar et al., 2024

exhibit much higher transdermal flux than human or animal skin, they are an excellent choice during the early stages of formulation development [21]. Synthetic membranes such as polydimethylsiloxane (PDMS), cellulose acetate membrane and Strat-M® have all been used as alternatives to *ex vivo* models for conducting *in vitro* skin permeation experiments. PDMS is easily available and has good batch-to-batch reproducibility, however, it has poor correlation with the human skin. Cellulose acetate (CA) membrane is simple and porous with no rate controlling property, hence it has largely been replaced with other membranes that are structurally similar to human skin such as Strat-M® membrane (Fig. 3). Strat-M® is approximately 300 µm thick with an outer layer which resembles the SC. This is supported by multiple layers of polyethersulfone mimicking the dermis, and then the innermost layer composed of polyolefin non-woven fabric. Studies have shown that the permeation of both hydrophilic and lipophilic drugs across Strat-M® membrane was comparable to that of human cadaver skin [22, 23].

3-D Printed Skin Models

Advancements in skin bioprinting technology have led to substantial improvements in therapeutic approaches [24]. This innovative technology also holds potential as a reliable platform for evaluating cosmetic and pharmaceutical formulations [25]. In recent years, 3D bioprinting has garnered substantial attention from researchers in the fields of medicine and pharmaceuticals. It is essential to distinguish between 3D printing and 3D bioprinting [26]. While 3D printing involves the layering of materials such as glass, polymers or rubber to create three-dimensional structures, it finds applications across diverse domains, including medicine, dentistry, and engineering. Specifically, in the medical field, it is used for producing anatomical models, implants and therapeutic devices [27]. Conversely, 3D bioprinting is a pioneering technique that uses cells and biomaterials to fabricate three-dimensional structures. Like 3D printing, bioprinting replaces conventional ink with bioink, enabling the production of tissue structures with remarkable reproducibility and precision. By using computer-controlled bioprinters, cells and biomaterials can be accurately deposited to form predefined structures [28].

Bioprinting typically involves three main stages. The first stage involves collecting information about tissues or organs to determine suitable materials and establish models. In the second stage, converting this information into electrical signals that guide the printer in fabricating tissues, and finally, developing stable structures [29]. This approach has demonstrated the capability to generate skin tissues using specific types of cells. In 2009, Lee and colleagues developed the earliest bioprinted skin constructs by embedding human dermal fibroblasts within a collagen hydrogel [30]. At the same time, Koch *et al*. contributed to the field by formulating skin equivalents using a collagen-based bioink containing both keratinocytes and fibroblasts [31]. Meanwhile, Binder *et al*. introduced 3D inkjet-printed skin substitutes incorporating human fibroblasts and keratinocytes, aimed at wound healing applications [32]. Since these initial breakthroughs, significant progress has been made in this field. For instance, Ng *et al*. developed a pigmented skin model where three distinct skin cell types-keratinocytes, melanocytes, and fibroblasts are used to create the three-dimensional colored human skin constructs [33].

Hou *et al*. developed a simpler version of artificial 3D skin model to screen nanoparticles intended for transdermal penetration. This model was created by printing blank collagen hydrogel and fibroblasts alternately, layer by layer. In order to verify the efficacy of this platform, silica nanoparticles with varying surface charges are evaluated for their penetration ability. The results show that positively charged nanoparticles have deeper penetration, which is in line with an observation made in a previous study using real skin tissue [34].

Revolutionary 3D bioprinting offers hope for overcoming organ shortages, yet its broader use is restricted by

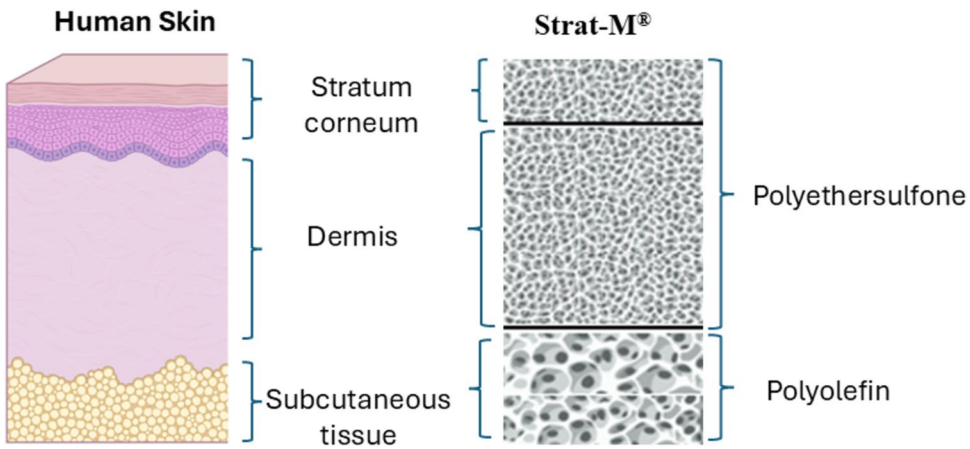

Fig. 3 Diagrammatic representation of a Strat-M® membrane showing the arrangement of different layers and its similarity with the human skin layers

technical challenges, material limitations, and substantial costs [27]. Reproducing the intricate architecture of human tissues and organs demands exceptional accuracy. Unlike conventional 3D printing, bioprinting functions at the cellular scale, necessitating the precise placement of living cells and layering is essential to replicate natural biological systems. Material challenges also play a significant role, with the development of suitable bioinks being a complex task. Bioinks must mimic the natural extracellular matrix to support cell viability and function and ensure the mechanical properties of bioprinted tissues match those of native tissues are essential for their functionality [35].

Regulatory oversight of bioprinting spans several domains, including medical devices, pharmaceuticals, and tissue engineering, which complicates the regulatory landscape. In the European Union, bioprinted tissues are regulated under the Advanced Therapy Medicinal Products (ATMP) framework, while in the United States, the FDA oversees the safety and efficacy of bioprinted products. Despite these efforts, the lack of standardized production methods and quality certifications for bioprinted tissues poses significant challenges in guaranteeing their safety and effectiveness. Addressing these complexities requires a careful balance between fostering innovation and maintaining ethical standards to ensure responsible development and application of bioprinting technologies. International bodies like ISO and ASTM International are actively working to create globally recognized standards for the production and evaluation of bioinks. Developing such unified guidelines will help harmonize regulatory procedures across different countries, facilitating global collaboration and improving market accessibility [36].

Skin Barrier Integrity Testing

The abdominoplasty procedure and the associated skin processing can potentially cause accidental damage to the stratum corneum. Therefore, it is necessary to check skin integrity to ensure that only undamaged skin samples are used for permeation experiments. One of the common test FDA suggests is the measurement of trans-epidermal water loss (TEWL) [37]. The TEWL test measures the rate of water vapor passing through the skin barrier. In this test, skin is positioned in a vertical diffusion cell with the SC side oriented toward the donor compartment, which is maintained at 32°C ± 1°C. After equilibration, the donor compartment may be removed carefully along with the skin to place the device probe directly on the skin surface, and at least three replicate measurements are recorded. Environmental control is critical for accurate TEWL readings, with ideal conditions at 21°C ± 2°C and 40–50% RH. A TEWL reading ≤ 15 g/m^2/hr is generally acceptable for human skin barrier integrity, while readings > 15 g/m^2/hr indicate compromised integrity [38].

Sample Variability in Skin Permeation Studies

Intra and inter-sample variability in skin permeation is a critical factor to consider when designing *in vitro* permeation tests using human skin [39]. Research indicates that abdominal skin tends to exhibit lower variability compared to other anatomical sites. Due to both inter-individual and intra-individual differences, aspects such as the selection of excised skin samples, the number of replicate experiments, and the number of donors used have garnered significant attention from researchers and regulatory bodies. According to EMA guidelines, inter-subject variability should be minimized through a crossover study design. The guideline also recommends using skin from at least 12 donors, with a minimum of two replicates per donor [40]. Meanwhile, the FDA's draft guidance on IVPT suggests conducting a pilot study with skin from 4 to 6 donors, using at least four replicate skin sections per donor. It is important to note that data from the pilot study should not be included in the statistical analysis of the pivotal IVPT study [41].

Diffusion Cell Assembly and its Design

Protocols for conducting *in vitro* permeation testing (IVPT) have been established by various regulatory bodies and organizations, such as the Organisation for Economic Co-operation and Development (OECD), the European Medicines Agency (EMA), and the United States Food and Drug Administration (FDA)[40–42]. Diffusion cells are commonly employed for conducting *in vitro* permeation experiments [43]. The two main types of diffusion cells are static and flow-through cells. The static type includes vertical upright Franz-type diffusion cells and side-by-side cells (Fig. 4). The selection of diffusion cell may depend on the type of formulation to be tested. A majority of permeation experiments are conducted using vertical static upright diffusion cells (VDC). The Franz diffusion cell is compliant with guidelines outlined in the USP [44]. The main advantage of vertical static upright diffusion cells is that different types of dosage forms can be tested including both solutions and semisolids. Though there exist different models, all have the same fundamental features which include donor and receptor compartments that are separated by a membrane. The permeant diffuses from the donor to the receptor and is then quantified using suitable analytical techniques. The receptor compartment is immersed in a thermoregulated water bath ensuring that the water level remains below the membrane. The receptor volume is kept constant and is constantly stirred using a magnetic stirrer throughout the experiment. This constant stirring and temperature control are necessary

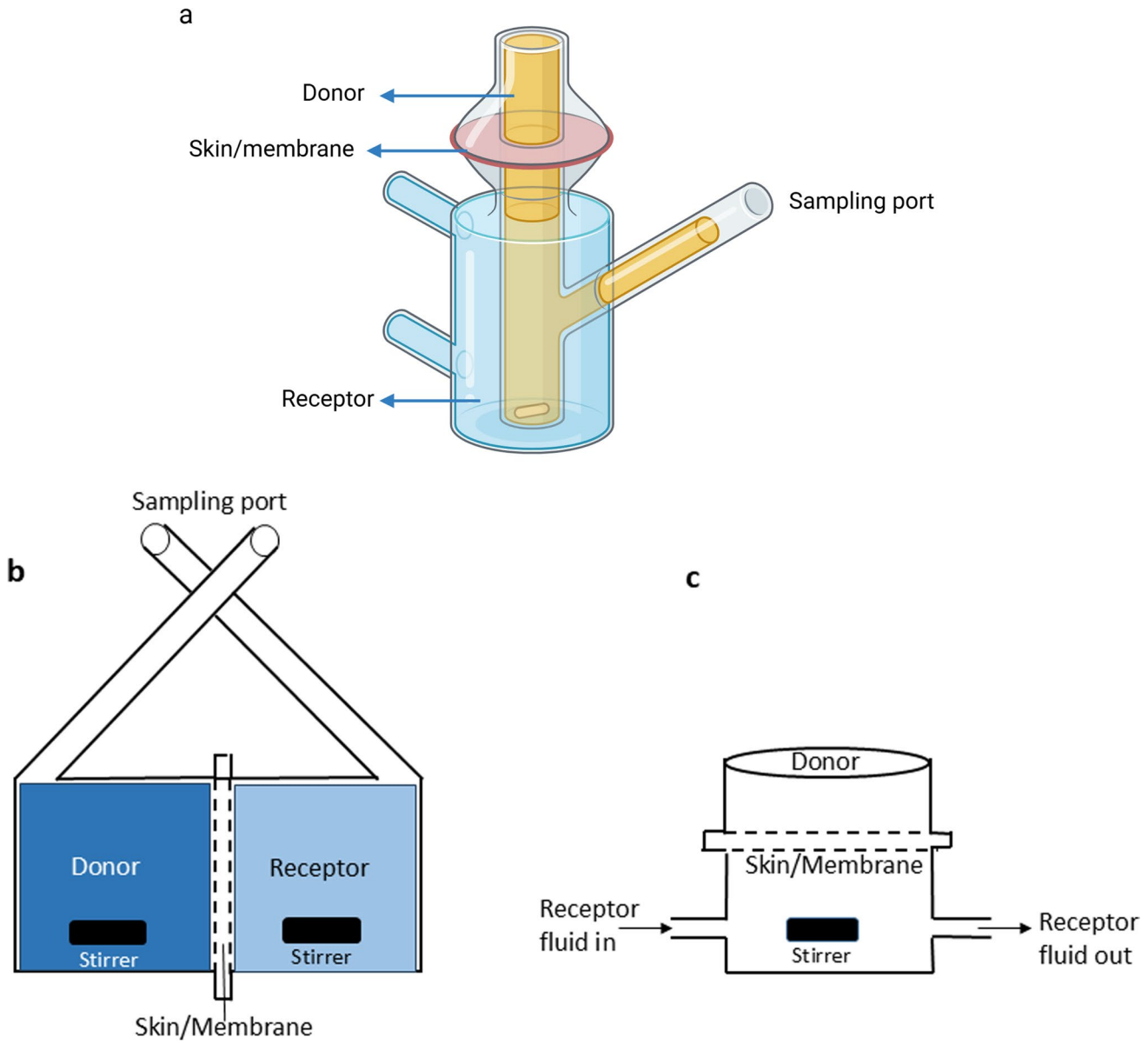

Fig. 4 Different types of diffusion cells for performing i*n vitro* permeation studies: **a** Static upright Franz-type diffusion cell, **b** Side by side cell, **c** Flow-through cell

for maintaining uniform drug distribution in the receptor compartment. A variant of this upright cell is the jacketed static diffusion cell, where a temperature-controlled water jacket surrounds the diffusion cell thereby avoiding any possibility of water entering the receptor chamber [45]. Another modification of the Franz-type diffusion cell is the side-by-side cell, wherein both compartments can be stirred, but these cells are limited to certain formulations only, such as finite dose preparations [39]. One of the major challenges associated with static diffusion cells is the maintenance of sink conditions. This is particularly an issue when the receptor chamber has a low volume, and this may lead to the loss of sink conditions. At the same time, slow permeating compounds coupled with a large receptor compartment volume may pose problems in drug detection. Thus, whenever static diffusion cells are used it is important to keep a very low receptor concentration to maintain the concentration gradient.

An alternative to the static cell approach is the use of flow-through cells (USP Apparatus 4) where the receptor fluid constantly flows through the cell at a speed of 1–2 mL/hr, mimicking blood flow within or beneath the skin [46]. This is achieved by connecting the receptor compartment to a peristaltic pump and this continuous flow of receptor fluids ensures that sink conditions are maintained throughout the study. The receptor fluid can be collected into a receiver tank

and analysed separately or by using an in-line flow-through analysis. Flow-through cells are more sophisticated and are more expensive than static diffusion cells. For those compounds that permeate faster, a higher flow rate is required in order to maintain the sink conditions, and this will increase the receptor volume. Conversely, when compounds with low fluxes are tested, a large volume of the circulating receptor fluid can cause problems in the detection of permeants [47].

Donor Phase Design

The way in which drug samples or formulations are applied to the skin is also crucial. The dose applied to the donor compartment is crucial in designing *in vitro* permeation experiments and mass balance studies. Two dosing scenarios are possible: 1) infinite dose, in which a large amount of formulation is applied, whereby a characteristic steady state can be obtained, and 2) finite dose, where a small formulation amount, such as ≤ 10 mg/cm^2, is applied, and a substantial reduction of the active is observed. Infinite doses are ideal for studying permeation enhancements or evaluating new actives but are not representative of *in vivo* conditions. Finite doses mimic *in vivo* scenarios better and are appropriate for assessing bioequivalence, especially with semi-solid dosage forms. The donor phase may also be occluded or left open.

Guidelines recommend dosing within 2–15 mg/cm^2, with un-occluded donor compartments to ensure reproducibility and uniform application [48]. The experimental protocol will be designed based upon the type of permeant/formulation under investigation or based on the intended application. Topical sprays and solutions are typically applied to the skin and allowed to dry, making non-occluded set-ups essential for simulating the evaporation of volatile solvents such as ethanol or isopropyl alcohol, which can significantly influence drug deposition and permeation. Similarly, non-occluded conditions are necessary for gel formulations, such as minoxidil or diclofenac gels, to evaluate the impact of solvent evaporation on drug delivery kinetics [49, 50]. Occluded donor phases and saturated formulations may be used in order to determine the maximum permeation potential of a substance as occlusion can enhance the permeation of compounds [51]. A supersaturated donor phase can compensate for depletion as a result of permeation, and this can be achieved by the addition of excess drug at regular intervals to maintain a thermodynamic equilibrium throughout. A steady state is achieved when the permeation is consistent and remains unchanged for a certain period. The time taken to reach steady state is largely dependent on the donor phase concentration and the permeability coefficient of drugs [52]. The amount of drug that permeates into the receptor solution should be assessed in each diffusion cell by calculating the cumulative total permeation over the course of the IVPT. This value can be represented as a percentage of the nominal drug content in the applied dose.

Receptor Phase Design

Drug Solubility in the Receptor Phase

The receptor phase is there to accept permeating drug molecules, as such, it essentially mimics the circulation *in vivo* [53]. Ideally, the concentration of the drug in the receptor phase should not exceed 10% of the drug's saturation solubility in order to help maintain sink conditions [54]. Thus, the selection of receptor fluid in which the permeant has sufficient solubility is extremely important for obtaining valid and reliable permeation data. Isotonic phosphate buffered saline (PBS pH 7.4) is often suggested as a suitable receptor fluid for *in vitro* experiments, however, highly lipophilic drugs may be poorly soluble in PBS 7.4. Such permeants typically possess high Log *P* values and extremely low water solubility, it is possible that they may permeate the skin/membrane but may not then readily diffuse into the receptor fluid. The solubility of permeants in the receptor phase can be enhanced by the addition of a solubilising agent or co-solvent to the receptor phase [55]. However, such solubility enhancers should cause no damage to the integrity of the membrane used else artificially high permeation data may be obtained. Studies have reported that co-solvents such as ethanol can disturb the skin integrity [53] and should be avoided in higher concentrations. For this reason, surfactants such as cetrimide, polysorbates or sorbitan monostearate are commonly employed instead [52, 56]. Surfactants are also capable of solubilising a variety of different drugs and quantification can usually be carried out without complex extraction procedures. It is recommended to use diffusion cells with controlled RPM for the homogenous drug mixing in the receptor compartment. At the same time, care should be taken to prevent excessive mixing that causes vortex formation and can compromise the integrity of membranes.

Drug Stability in the Receptor Phase

The stability of permeants upon exposure to receptor media and skin extract is crucial for estimating cumulative drug permeation and flux. Skin extracts can be prepared by mincing and macerating the tissue in the receptor medium with continuous stirring. The extract is then treated with the drug, and the drug content can be analysed by withdrawing specific volume of samples at predetermined time points. In our previous study we evaluated the stability of tocotrienol and curcumin in PBS containing 1% tween 80 and as well as human skin extracts at 37°C [57]. Polysorbates are known to enhance the stability of hydrophobic drugs through

micelle formation, supporting its use in diffusion media [58]. To further support this, another report has shown that PBS (pH 6.8) containing 1% Tween 80 had retained ˃95% of curcumin concentration at the end of a 24-h study suggesting the stability of curcumin in the presence of Tween 80 [59]. Tocotrienol showed an improved stability in media containing skin extract compared to media alone possibly due to ionic interactions or hydrogen bonding between the drug and skin components. Protonated groups in tocotrienols may interact with negatively charged skin proteins, forming ionic linkages and crosslinks that enhance tocotrienols thermal stability [60]. Furthermore, it is suggested to add an antimicrobial agent in the receptor solution (0.1% sodium azide or 0.01% gentamicin sulfate) to prevent bacterial growth in the diffusion cell, irrespective of the study duration.

Importance of Temperature Control

Temperature at which an *in vitro* permeation experiment is conducted is important since this can have an impact on drug solubility and diffusion. The experimental temperature is usually maintained at 37°C to simulate physiological conditions and this will typically mean that the temperature of the skin surface is usually around 32°C. Most reported transdermal permeation experiments are usually conducted between 32 to 37°C [55]. It is important to maintain a constant temperature throughout the experiment as a small change in the temperature can cause significant fluctuations in permeation [61]. Temperature-controlled water baths are frequently employed to regulate the diffusion cell temperature. Studies report that transdermal flux increases by two-fold with a 10°C rise in temperature [62]. In addition, the ambient laboratory temperature and humidity should be monitored and recorded during the study. It is recommended to maintain an environmentally controlled temperature range of 21°C ± 2°C at 50% ± 10% relative humidity.

Sampling Techniques and Analysis

The volume of the sample withdrawn and the sampling interval are important for maintaining sink conditions. For small-volume receptor compartments, withdrawing large volumes leads to rapid dilution, while withdrawing too small volumes may not be sufficient for quantification, especially since the withdrawn samples will be further diluted with solvents before analysis. Ideally, for a 2-mL receptor volume, withdrawing a sample volume of 100–200 µL should be sufficient for quantification, provided that the developed analytical method is sensitive enough. Since synthetic membranes are expensive and biological membranes are difficult to harvest, it would be ideal to conduct trial runs to predict the volume required for analysis. The study duration and sampling interval depend on the permeability coefficient, which measures the rate at which a drug permeates through a membrane under a concentration gradient. For rapidly diffusing drugs, a shorter duration is sufficient to achieve a steady state. The FDA recommends using at least eight non-zero sampling time points, whereas the EMA requires a minimum of six [57, 63].

Choosing an appropriate analytical technique is critical due to the sensitivity required for detecting low permeant concentrations. Full-thickness human skin or animal skin serves as a robust barrier, often resulting in low permeation rates and, consequently, low drug concentrations in receptor media [64]. High-Performance Liquid Chromatography (HPLC) is the most widely employed analytical technique due to its high sensitivity, specificity, and reproducibility [65]. Fluorescence detectors (FLD) in HPLC are highly sensitive can detect analytes at nanogram to picogram levels, often more sensitive than UV or Photodiode Array Detector (PDA) detectors. However, HPLC requires meticulous method development and validation, and it may face interference from excipients or skin components, especially when using UV detectors [66]. For drugs present in very low concentrations or in complex biological matrices, Liquid Chromatography–Mass Spectrometry (LC–MS) offers superior sensitivity and selectivity. It is highly effective for low-level quantification and pharmacokinetic studies, but it is expensive, requires skilled operation, and may be affected by matrix-induced ion suppression. Gas Chromatography (GC), often coupled with mass spectrometry (GC–MS), is suitable for volatile and thermostable compounds and offers excellent separation efficiency. Nevertheless, it is not ideal for non-volatile or thermolabile drugs, and derivatization steps are often needed, which can complicate sample preparation. On the other hand, UV–Vis spectrophotometry, while simple, rapid, and cost-effective, is limited by its relatively low sensitivity and specificity. It is best suited for drugs with strong UV absorbance and minimal formulation interference but is generally inadequate for low-concentration samples. Overall, each method presents a balance between sensitivity, complexity, and suitability depending on the drug's physicochemical properties and nature of the sample being analysed.

Drugs and Therapeutic Areas where IVPT have Demonstrated Importance

Table I Selected Drugs and Therapeutic Applications Utilizing IVPT, along with Formulation Types and Skin or Membrane Models

Drugs	Therapeutic area	Formulation type	Skin/membrane	Reference
Diclofenac	NSAIDs	Emulsion, gel, ointment, solution, patch	Cellulose acetate	[67, 68]
Ketoprofen	NSAIDs	Cream, gel, solution	Strat-M®	[69]
Fentanyl	Opioid Analgesic	Patch	Porcine/human skin	[70]
Capsaicin	Analgesic	Patch, emulsion	HSE/polyethersulfone	[71]
Flurbiprofen	NSAIDs	Nanoparticle gel	Human skin	[72]
Ibuprofen	NSAIDs	Gels/plasters	Human skin	[73]
Lidocaine	Local anaesthetic	Gel/ointment	Cellulose acetate	[74]
Nitroglycerin	Anti-anginal	Patch	Human skin/Rat abdominal skin	[75, 76]
Nifedepine	Antihypetensive	Patch	Porcine skin	[77]
Estradiol	Hormone replacement therapy (HRT)	Microemulsions/patch	Human live/cadaver skin	[78, 79]
Testosterone	HRT	Gel	Strat-M®	[80]
Nicotine	Smoking cessation	Patch	Regenerated cellulose	[81]
Salicylic acid	Keratolytic	Gel	Porcine skin	[82]
Acyclovir	Topical antiviral	Cream	Human skin	[83]
Tretinoin	Acne	Deformable vesicles	Pig ear skin	[84]
Fluconazole	Antifungal	Transferosomal gel	Porcine skin	[85]
Selegiline	Parkinson's disease	Transdermal film	Dialysis membrane	[86]
Rasagilene	Parkinson's disease	Microemulsion gel	Rat abdominal skin	[87]
Rotigotine	Parkinson's disease	Microemulsion	Rat abdominal skin	[88]
Calcipotriol	Psoriasis	Ointment	Human skin	[89]
Scopolamine	Motion sickness	Patch	Abdominal porcine skin	[90]
Minoxidil	Hair loss treatment	Transferosomal gel	Porcine skin	[91]
Permethrin	Scabies	Cream	PVDF membrane	[92]

HSE: Heat Separated Epidermis; NSAIDs: Nonsteroidal anti-inflammatory drugs

Conclusion

The success of *in vitro* permeation studies in topical and transdermal drug delivery research hinges on meticulous attention to experimental design. Key elements such as the selection of an appropriate skin model, careful consideration of receptor phase composition to ensure drug solubility and stability, precise temperature control, optimal stirring speed, reliable sampling and validated analytical techniques collectively influence the reliability and reproducibility of permeation data. Adopting standardized and reproducible protocols while tailoring them to the physicochemical properties of the drug ensures meaningful data that bridges the gap between *in vitro* experiments and clinical applications. Future directions should focus on improving predictive models and validating *in vitro* findings with *in vivo* correlations, thereby advancing the development of efficient and patient-friendly topical and transdermal drug delivery systems.

Author Contributions Rajesh Sreedharan Nair contributed to the conception, acquisition of data and drafting the manuscript. Andrew Morris and Nashiru Billa contributed to the conception and revising the manuscript.

Declarations

Conflict of interest Authors declare no conflicts of interest.

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