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INVITED REVIEW

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In vitro and *ex vivo* experimental models for evaluation of intranasal systemic drug delivery as well as direct nose-to-brain drug delivery

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Abstract

The intranasal route of administration provides a noninvasive method to deliver drugs into the systemic circulation and/or directly into the brain. Direct nose-to-brain drug delivery offers the possibility to treat central nervous system diseases more effectively, as it can evade the blood–brain barrier. *In vitro* and *ex vivo* intranasal models provide a means to investigate physiological and pharmaceutical factors that could play a role in drug delivery across the nasal epithelium as well as to determine the mechanisms involved in drug absorption from the nose. The development and implementation of cost-effective pharmacokinetic models for intranasal drug delivery with good *in vitro-in vivo* correlation can accelerate pharmaceutical drug product development and improve economic and ecological aspects by reducing the time and costs spent on animal studies. Special considerations should be made with regard to the purpose of the *in vitro*/*ex vivo* study, namely, whether it is intended to predict systemic or brain delivery, source and site of tissue or cell sampling, viability window of selected model, and the experimental setup of diffusion chambers. The type of model implemented should suit the relevant needs and requirements of the project, researcher, and interlaboratory. This review aims to provide an overview of *in vitro* and *ex vivo* models that have been developed to study intranasal and direct nose-to-brain drug delivery.

KEYWORDS
ex vivo experimental models, experimental models, *in vitro* experimental models, intranasal drug delivery, nose-to-brain drug delivery

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1 | INTRODUCTION

Intranasal (IN) drug delivery is an attractive, noninvasive route of administration for delivering drugs into the systemic circulation, but also provides a route for drug delivery directly into the brain. Nose-to-brain (N2B) drug delivery presents several advantages over other routes of drug administration such as bypassing gastric enzymatic and chemical degradation, the blood–brain barrier (BBB), as well as hepatic first-pass metabolism (Du et al., 2006). The nasal epithelium is well-vascularized and relatively highly permeable, which facilitated rapid drug absorption (Figure 1) (Ladel et al., 2019; Mittal et al., 2014). Furthermore, IN delivery offers painless and easy self-administration, as it does not require sterile dosage forms nor specialized administration techniques (Pund et al., 2013).

2 | ANATOMY OF HUMAN NASAL CAVITY

The internal nose is located between the base of the skull and the roof of the mouth (Pires et al., 2009; Sosnik, 2015). The nasal cavity extends roughly 12–14 cm in length and 5 cm in height, providing a combined surface area of 150–200 cm^2 and a total volume of 13–25 mL (Bourganis et al., 2018). The relatively large internal surface area can be ascribed to the presence of three nasal conchae, as well as microvilli on certain epithelial cells (Salade et al., 2019). The cavity of each nostril can be sectioned into four anatomical regions: the nasal vestibule, atrium, respiratory, and olfactory area (Figure 1) (Du et al., 2006).

The respiratory region covers the lateral walls of the nasal cavity and the three protruding conchae, which make up the largest part of the nasal cavity (about 130 cm^2). The respiratory region (Figure 1B) is

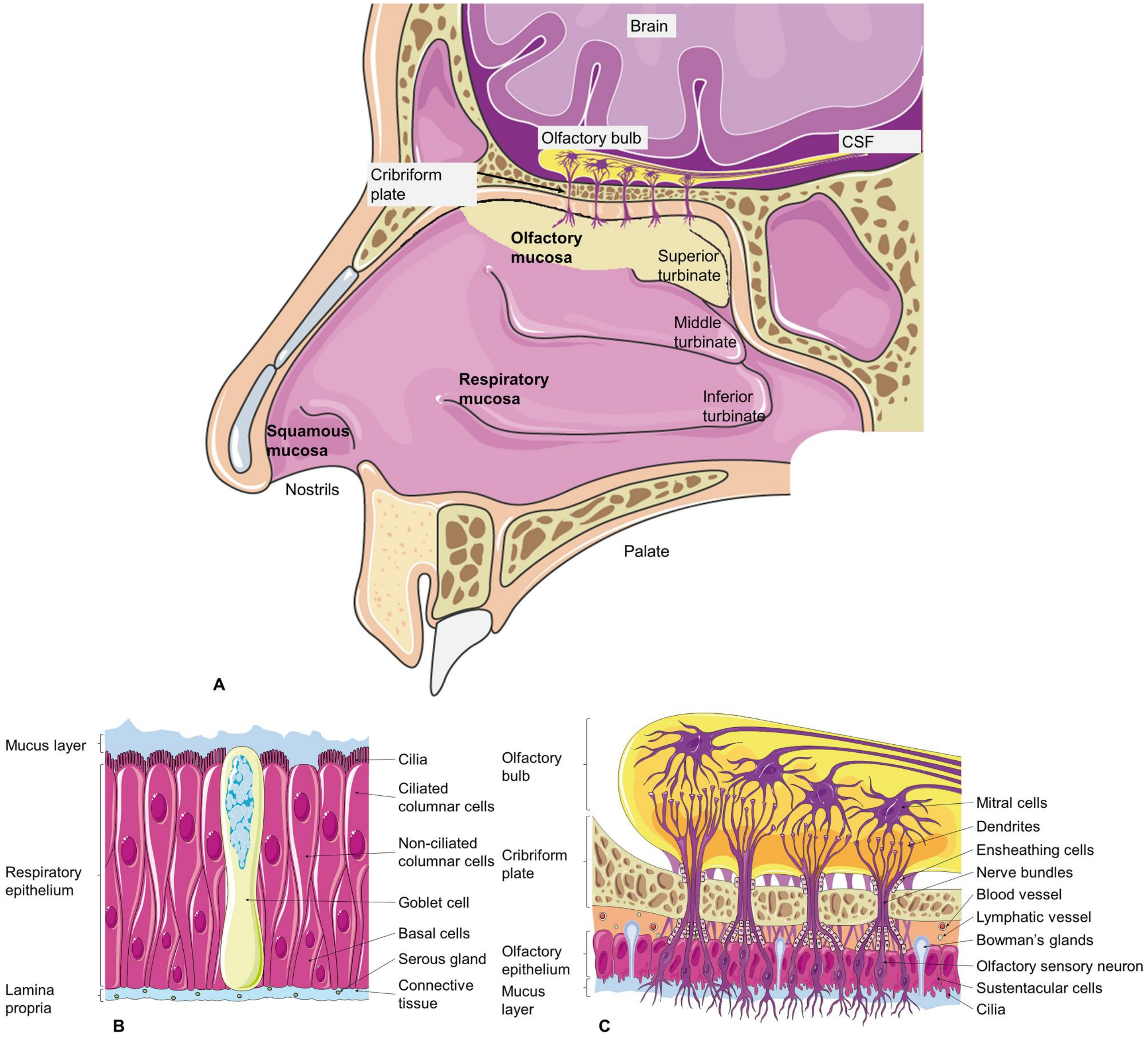

FIGURE 1 Schematic illustration of the anatomy of the human nasal cavity. (a) Different areas in the human nasal cavity, (b) Composition of the respiratory epithelium, and (c) Composition of the olfactory epithelium (Illustrations modified with text from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License).

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covered in microvilli and highly vascularized, as it receives its blood supply from an arterial branch of the maxillary artery (Bourganis et al., 2018; Erdő et al., 2018), providing access to systemic circulation (Pires et al., 2009).

The olfactory region (Figure 1C) is located in the superior, dorsal part of the nasal cavity covering the inferior surface of the cribriform plate of the ethmoid bone. The region extends and bilaterally covers a partial area of the nasal septum, lateral wall, and superior conchae (Bourganis et al., 2018). This region covers ~10 cm^2 of the nasal cavity (Crowe et al., 2018). The cribriform plate is innervated with neurites, connecting the nasal cavity to the olfactory bulb in the brain (Bahadur & Pathak, 2012; Mistry et al., 2009).

To introduce a new substance for nasal administration, biopharmaceutical studies to obtain pharmacokinetic data such as membrane permeation is required. Different *in vitro* and *ex vivo* models have been developed to study drug permeation across the nasal mucosa, which include the use of immortalized epithelial cells, the use of primary cultured cells obtained from human, pig, or rat, and the use of excised nasal epithelial tissues from animals (e,g, cattle, sheep, pig, or rabbit) (Wengst & Reichl, 2010).

3 | MODELS TO DETERMINE INTRANASAL SYSTEMIC AND NOSE-TO-BRAIN DRUG DELIVERY

Several types of models can be implemented to study IN and direct N2B drug delivery, which include *in vivo* models, *in situ* perfusion models, *in vitro* cell culture models, *ex vivo* models, and *in silico* models (Agu & Ugwoke, 2008; Kim, 2008).

The most accurate method to determine the pharmacokinetics of a substance after IN administration is by means of *in vivo* models because they take the complex interplay of different physiological factors into account. Whole animal studies are, however, limited by factors such as cost and ethics-related concerns. To regulate the use of animals in research, the “Three R's Principle” was developed (Cabrera-Pérez et al., 2016). This principle is centered around reducing the number of animals used in research, implementing and refining scientific procedures to minimize pain, and replacing animals with alternative models where possible (Cabrera-Pérez et al., 2016). Subsequently, alternative models to investigate the pharmacokinetics of drugs such as membrane permeation properties have been developed to replace whole animals, which include tissue-based models, cell-based models, and cell-free models (Berben, Brouwers, et al., 2018; Westerhout et al., 2014).

In situ perfusion studies are considered the closest to *in vivo* studies, as they involve the investigation of drug delivery on an organ that remains part of the animal. With *in situ* studies, blood flow and innervation are retained; however, the model requires the use of whole animals that are put under anesthesia and the assays often require surgery, which renders it a low-throughput model with relatively high cost (Agu & Ugwoke, 2008; Volpe, 2010).

Cell-based *in vitro* studies for IN drug delivery studies include the use of epithelial cell monolayers grown from the RPMI 2650 cell line (human nasal epithelium), Calu-3 cell line (human lung carcinoma), or 16HBE14o cell line (human normal bronchial epithelium). These cell-based epithelial models can be used to investigate drug transport pathways from the nasal cavity across the epithelium, such as transcellular and paracellular passive diffusion, active transport, and active efflux transport, while it also supports mechanistic studies. With cell-based models, there is a risk of interlaboratory variability due to differences in culture conditions, labor-intensive procedures, a relatively low expression of certain active transporters, and the lack of a mucus layer (Volpe, 2010).

Ex vivo tissue-based studies have been developed as alternatives to whole animal or cell-based models. Within the context of biopharmaceutical drug delivery studies, the term *ex vivo* model refers to the use of tissues that have been excised from animals specifically for the purpose of experimental research (Agu & Ugwoke, 2008). Excised epithelial tissues retain architectural and anatomical regional properties. Tissues can be excised from humans or animals and supports the investigation of mechanistic and directional drug transport. However, *ex vivo* models face limited viability as a function of time and data can be influenced by suboptimal stirring conditions (Volpe, 2010).

In silico or computational determination of drug pharmacokinetics is based mostly on the physicochemical properties of the drug molecule such as pKa, molecular weight, solubility, and dissolution rate, which can easily oversimplify the complex process behind drug permeation across biological membranes (Wu et al., 2020).

3.1 | *In vitro* cell models for predicting intranasal and nose-to-brain drug delivery

In vitro cell models are considered important tools that can be used to investigate membrane permeability of candidate drug molecules as well as to investigate strategies that can be used to improve drug delivery. The purpose of an IN drug delivery model is to closely mimic the human nasal mucosal barrier and it should contain all the important physiological properties that may have an impact on drug pharmacokinetics after IN administration. Cell models offer advantages such as high-throughput screening, experimental control of the culture conditions, elucidating drug transport mechanisms, and measuring the effect of absorption-enhancing interventions on drug delivery (Mercier et al., 2019; Sousa & Castro, 2016). Different cell models are available to study IN drug delivery, which include primary cell cultures, immortalized cell cultures, and commercially available alternative cell-type models as comprehensively summarized in Tables 1–3 (Dimova et al., 2005; Furubayashi et al., 2020; Schmidt et al., 1998; Sousa & Castro, 2016).

3.1.1 | Primary cell cultures

Primary nasal epithelial cells are cultured *in vitro* after being harvested from live organisms such as humans, rats, or pigs. The high proliferation capacity and the genetic homogeneity of these cells give

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TABLE 1 Summary of selective studies on primary cell cultures used for *in vitro* intranasal permeation and cytotoxicity experiments.

Type of cells	Source/origin	Applications	Culture conditions	Growth media	Integrity	Permeation media	Duration of study	References
Pig respiratory cells	Harvested from ventral nasal concha	Permeation	ALI: Seeding density 1×10^5 cells/insert in ThinCert membranes, used 21 days after seeding	DMEM:F12 (1:1), 10% FBS, 2 mM Gln, 1% NEAA, 0.4 U/mL Penicillin –0.4 μg/mL Streptomycin, 0.6 I.U Gentamycin sulphate	TEER = 846 ± 550 Ω.cm^2	MEM without phenol red and PBS	Permeation period was for 24 h	Ladel et al., 2019
Pig olfactory cells	Harvested from the dorsal part of the dorsal nasal concha and the middle nasal concha	Permeation	ALI: Seeding density 1×10^5 cells/insert in ThinCert membranes, used 21 days after seeding	DMEM:F12 (1:1), 10% FBS, 2 mM Gln, 1% NEAA, 0.4 U/mL Penicillin –0.4 μg/mL Streptomycin, 0.6 I.U Gentamicin sulphate	TEER = 648 ± 371 Ω.cm^2	MEM without phenol red and PBS	Permeation period was for 24 h	Ladel et al., 2019

Abbreviations: ALI, air-liquid interface; DMEM, Dulbecco's modified Eagle's medium; DMEM:F12, Dulbecco's modified Eagle's medium/Nutrient Mixture F:12; FBS, fetal bovine serum; Gln, glutamine; MEM, minimal essential medium; NEAA, nonessential amino acids; PBS, phosphate-buffered saline; TEER, transepithelial electrical resistance.

them an advantage over immortalized cell lines (Mercier et al., 2019). Some difficulties associated with primary cell cultures include the limitation on the number of cells that can be obtained from a donor, ethical aspects related to obtaining human tissues (Kim, 2008), the dependency on repeated sampling of cells, limited lifespan, complex isolation, and cultivation of tight junction-forming cell layers (Dimova et al., 2005; Wengst & Reichl, 2010).

Selective studies using primary cell cultures used in drug permeation studies to predict nasal drug delivery are summarized in Table 1 in terms of the type of cells, the source or origin, applications, culture conditions, growth media, and integrity.

Ladel et al. (2019) evaluated and characterized porcine respiratory and olfactory primary epithelial cells against the RPMI 2650 cell line. In addition, they obtained similar results for transepithelial electrical resistance (TEER), mucus production, and ciliogenesis when comparing porcine and human primary epithelial cells with each other. This indicated that this primary cell model is an appropriate model to evaluate certain dosage forms. They have specified that it is important to use primary cells that originate from the relevant tissue type to study IN permeation.

3.1.2 | Immortalized nasal cell lines

Immortalized cell lines offer certain advantages, such as high proliferation capacity, high reproducibility, better cost-effectiveness, and easier maintenance when compared to primary cell cultures (Sousa & Castro, 2016). Due to the weak differentiation ability of immortalized cell lines, it may differ slightly from human nasal epithelial cells in terms of biophysiology. However, some *in vitro* models have been modified to become more complex and evolved from submerged monocultures to three-dimensional epithelia that is cultured under air–liquid interface (ALI) conditions (Mercier et al., 2019). The immortalized cell lines that are most often used to predict nasal drug permeation include 16HBE14o, Calu-3, Caco-2, and RPMI 2650 cell lines (Sousa & Castro, 2016; Wang et al., 2019).

Selective studies on immortalized cell cultures used in drug permeation studies to predict nasal drug delivery are summarized in Table 2 in terms of the cell line, applications, culture conditions, growth media, and integrity. The results from some of the studies are briefly discussed below.

The characteristics of five cultured cell models that are commonly used to study nasal drug absorption were compared, which included EpiAirway, MucilAir, Caco-2, Calu-3, and Madin-Darby canine kidney (MDCK) cells. The model drugs used to investigate the paracellular, transcellular, and passive or active transport were inulin, atenolol, sulfanilic acid, mannitol, acyclovir, quinidine, antipyrine, and methotrexate. Of the five cell lines, the barrier function of Caco-2, Calu-3, and MucilAir was relatively similar to that of the rat nasal epithelial tissue. This indicated that these cell lines could be used for the estimation of drug absorption from the nasal cavity of the rat (Furubayashi et al., 2020).

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TABLE 2 Summary of selective studies on immortalized cell lines used for *in vitro* intranasal permeation and cytotoxicity experiments.

Cell line	Application	Seeding and culture conditions	Growth media	Integrity/viability	Study media	Duration of study	References
16HBE14o	N2B: Cellular uptake and cytotoxicity	24-well plate, 5×10^4 cells/well for cellular uptake and 10^4 cells/well in a 96-well plate for cytotoxicity assessment	Not specified	Not specified	Not specified	24 h exposure for cytotoxicity and up to 2 h for cellular uptake	Tang et al., 2019
16HBE14o	N2B: Cellular uptake and cytotoxicity	Seeding density of 5×10^3 cells/well in a 96-well plate for cytotoxicity, and a seeding density of 2×10^4 cells/well for cellular uptake	Not specified	Not specified	Not specified	24 h exposure for cytotoxicity and up to 2 h for cellular uptake	Meng et al., 2018
Caco-2	Permeation	Seeding density of 1.8×10^5 cells/well and used 17–20 days after seeding	DMEM, 10% FBS, 1% L-glutamine, 1% NEAA and 0.5% antibiotic-antimycotic solution	TEER = 771 ± 8 $\Omega.cm^2$	Not specified	Not specified	Furubayashi et al., 2020
Caco-2	Cytotoxicity and permeation	Cytotoxicity: Seeded in 96-well plates at a seeding density of 5×10^3 cells/well and used at approximately 80% confluence Permeation: Seeded in Transwell plates (1.13 cm^2) at a seeding density of 2×10^5 cells/well and used after 21 days	DMEM supplemented with 20% FBS, 1% L-glutamine and 1% penicillin/streptomycin	LY transport (for permeation study) < 3% at the end of the permeation experiments	HBSS/HEPES	Cytotoxicity exposure was for 2 h Permeation study period was 2 h	Cirri et al., 2021
Calu-3	IN: Permeation and cytotoxicity (MTS/PES assay),	Used between passages 23–28 Permeation: Seeded in 12-well plates with a seeding density of 2×10^5 cells/well, 2 days after seeding cells were grown under ALI conditions Cytotoxicity: Seeded onto 96-well plates at a seeding density of 5×10^3 cells/well	DMEM:F12 (no supplements were specified)	TEER >500 $\Omega.cm^2$ for the permeation study	HBSS (pH 7.4)	Permeation study period was 150 min Exposure for cytotoxicity was 4 h	Qian et al., 2018

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TABLE 2 (Continued)

Cell line	Application	Seeding and culture conditions	Growth media	Integrity/viability	Study media	Duration of study	References
Calu-3	Permeation and cytotoxicity (LDH cytotoxicity)	Permeation and cytotoxicity: Passages used between 31 and 34. Seeded in 24-well plates (0.7 cm^2) at a seeding density of 2×10^5 cells/cm^2, grown for 1 day and cultured ALI and used after 3 weeks	A-MEM supplemented with 2.5% FBS and 2% GlutaMAX	Only cells with >275 $\Omega.cm^2$ TEER values on the study day were used for permeation study, following sample collection only wells with TEER values >250 $\Omega.cm^2$ were considered to maintain integrity, as well as LY $P_{app} \leq 1 \times 10^{-6}$ cm/s after 1 h incubation after permeation study	HBSS/0.01 M HEPES with and without 4% BSA	Permeation study period was 4 h Cytotoxicity samples were taken after permeability study	Sibinovska et al., 2022
MDCK	IN: Bidirectional permeation	Seeded in 24-well plates, used after confluent monolayer formed after 3–5 days	Culture media: HBSS with 25 mM HEPES and 25 mM glucose	TEER was measured 10 min after culture media added and after permeation study, but no range is given	Not specified (experiments conducted at two pH levels, i.e. apical and basolateral at pH 7.4, and pH 5.5 on the apical side and pH 7.4 on the basolateral side)	Permeation study period was 2 h	Krieter et al., 2019
MDCK	Permeation	Seeding density of 1.8×10^5 cells/well and used 6–7 days after seeding.	Modified α-Eagle medium, 10% FBS and 0.5% antibiotic-antimycotic solution	TEER = 2120 ± 45 $\Omega.cm^2$	HBSS supplemented with 15 mM glucose and 10 mM HEPES (pH 7.4)	Permeation study period was 60 min	Furubayashi et al., 2020
MDCK II and MDCK-BCRP	N2B: Cytotoxicity (Alamar blue assay), cellular uptake and bidirectional permeation	Cytotoxicity: Seeded onto 96-well at a seeding density of 1×10^4 cells/well and used after 24 h Cellular uptake (MDCK-BCRP cells only): Seeded in 12-well plate at a seeding density of 3×10^5 cells/well and used after 48 h Permeation: Seeded in 12-well plates at a seeding density of 6×10^5 cells/well and used after 7 days when intact monolayer was formed	DMEM supplemented with 0.04 M sodium bicarbonate, 10% FBS and 1% penicillin/ streptomycin	Permeation: Intact monolayers considered when TEER >90 $\Omega.cm^2$	Cytotoxicity: Blank medium Cellular uptake: HBSS/ 10 mM HEPES (pH 7.4)	Cytotoxicity exposure for 24 h Cellular uptake exposure for 30 min Permeation study period was for 2 h (under gentle agitation)	Gonçalves et al., 2021
RPMI	IN: Permeation (bidirectional, thus	LCC: Cells used between passages 29–41. Seeded in a 24-well plate at a seeding	A-MEM, 2% GlutaMAX and 2.5% FBS	LCC: TEER = 20 $\Omega.cm^2$ After permeation TEER >17 $\Omega.cm^{2-}$ and LY	HBSS/0.01 M HEPES (with or without 1% DMSO)	Permeation study period was 3 h	Sibinovska et al., 2019

(Continues)

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TABLE 2 (Continued)

Cell line	Application	Seeding and culture conditions	Growth media	Integrity/viability	Study media	Duration of study	References
	ABC transporter functionality studies)	density of 2 × 10^5 cells/cm^2 and used 3 weeks after seeding. ALI: Culture medium was removed 2–3 days after seeding, and then grown for 3 weeks.		P_{app} ≤ 11 × 10^{-6} cm/s. ALI: TEER = 34 Ω.cm^2 After permeation TEER >25 Ω.cm^2 and LY P_{app} ≤ 7 × 10^{-6} cm/s.			
RPMI	Permeation and cytotoxicity (LDH cytotoxicity)	Permeation and cytotoxicity: Passages used between 31 and 34. Seeded in 24-well plates (0.7 cm^2) at a seeding density of 2 × 10^5 cells/cm^2, grown for 1 day and cultured ALI and used after 3 weeks	A-MEM supplemented with 2.5% FBS and 2% GlutaMAX	Only cells with >25 Ω.cm^2 TEER values on the study day were used for permeation study, following sample collection only wells with TEER values >30 Ω.cm^2 were considered to maintain integrity, as well as LY P_{app} ≤ 1 × 10^{-5} cm/s after 1 h incubation after permeation study	HBSS/0.01 M HEPES with and without 4% BSA	Permeation study period was 4 h Cytotoxicity samples were taken after permeability study	Sibinovska et al., 2022
PC-12 neural like cells	Cell viability (with MTT assay)	Not specified	RPMI 1640 media, 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin	Nonpermeation study	Not specified	Exposure was for 24 h	Arisoy et al., 2020
PC-12 neural like cells	Cell viability (with MTT assay)	Passage 12–25	DMEM:F12 with 10% HS, 5% FBS, and 1% penicillin/streptomycin solution	Nonpermeation study	HBSS	Exposure was for 15,30,60 min and 24 h	Rassu et al., 2020

Abbreviations: ABC, ATP-binding cassette; ALI, air–liquid interface; A-MEM, advanced minimum essential medium; BCRP, breast cancer resistance protein; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; DMEM:F12, Dulbecco's modified Eagle's medium/Nutrient Mixture F:12; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; HBSS, Hank's balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HS, horse serum; IN, intranasal; LCC, liquid-covered culture; LDH, lactate dehydrogenase; LY, Lucifer yellow; MDCK, Madin-Darby canine kidney; MTS/PES, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt]/phenazineethosulfate; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; N2B, nose to brain; NEAA, nonessential amino acids; TEER, transepithelial electrical resistance.

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TABLE 3 Commercially available cell models used for *in vitro* intranasal permeation and cytotoxicity experiments.

Cells	Applications	Culture conditions	Growth media	Integrity/viability	Transport media	Duration of study	References
EpiAirway EPI-606-X	Permeation	Not specified	EpiAirway culture assay media with 4% bovine serum albumin	LY stayed the same at 18.5 h than at T_0	Not specified	Not specified	Berger et al., 2020
EpiAirway	Permeation	ALI	Assay medium for EpiAirway (serum free and contains growth factors, phenol red and penicillin/streptomycin)	TEER = 391 ± 50 Ω.cm²	Not specified	Not specified	Furubayashi et al., 2020
MucilAir	IN: Permeation (bidirectional)	Grown in a 24-well plate under ALI	Commercially available serum-free culture medium (Purchased from Epithelix)	Not specified	HBSS/0.01 M HEPES (with or without 1% DMSO)	Permeation study period was 4 h	Sibinovska et al., 2019
MucilAir	Permeation (as well as bidirectional)	Not specified	MucilAir (Epithelix) culture medium.	TEER = 316 ± 31 Ω.cm² (TEER>200 Ω.cm² was used in permeation experiments)	Not specified	Not specified	Mercier et al., 2019
MucilAir	Permeation	ALI	Culture medium for MucilAir (serum free and contains growth factors, phenol red and penicillin/streptomycin)	TEER = 560 ± 34 Ω.cm²	Not specified	Not specified	Furubayashi et al., 2020

Abbreviations: ALI, air–liquid interface; DMSO, dimethyl sulfoxide; HBSS, Hank's balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LY, Lucifer yellow; TEER, transepithelial electrical resistance.

The Calu-3 and RPMI 2650 cells were cultured under ALI to determine if these cell models have the ability to detect differences in drug absorption from different types of formulations such as solution- and suspension-based formulations. Both these cell models demonstrated a sufficient ability to distinguish and to show the effect of formulation composition on the permeability of various drugs. Sibinovska et al. (2022) concluded that these cell lines can be applied to investigate the permeation of drugs that is administered IN, for both local and systemic effects, and from solution- or suspension-based formulations.

The MDCK cell line can be transfected with human ABCG2, in order for the cell line to express breast cancer resistance protein (BCRP) to investigate the influence of the BCRP transporter on the permeation of different drugs. Gonçalves et al. (2021) employed the MDCK-BCRP cells, alongside the parental MDCK cells to conduct bidirectional permeation studies with three antiepileptic drugs (lacosamide, zonisamide, and levetiracetam) to determine the influence of the BCRP transporter on the permeation of the selected drugs. This study revealed that lacosamide inhibit BCRP, while levetiracetam did not exhibit activity as a substrate or an inhibitor, whereas zonisamide was identified as a BCRP substrate, because the addition of Ko143 (a BCRP inhibitor) decreased the flux ratio by 53.9%. Zonisamide was administered via IN and intravenous (IV) to mice in a preclinical *in vivo* study. After IV administration of zonisamide (with and without elacridar, a known P-glycoprotein [P-gp] and BCRP modulator), it was found that the presence of elacridar increased the brain exposure three-fold, while leaving the plasma concentration unchanged. This suggested that BCRP played a role in the transport of zonisamide through the BBB. On the other hand, after the IN administration of the zonisamide, no changes were observed in the plasma concentration or the brain exposure, showing that the influence of the BCRP transporter was less applicable than for IV administration. A possible explanation for this is uptake via the direct N2B transport pathway after IN administration, which indicated that the IN administration of antiepileptics is a relevant strategy to bypass the BBB.

The suitability of the RPMI 2650 cell line for drug permeation studies was investigated by studying the permeation of 23 model compounds, several zero-permeability markers, as well as P-gp and BCRP transporter substrates and inhibitors. The RPMI 2650 cells were cultured in ALI and liquid–liquid interface (LLI), and the permeation results correlated with that found across the fully differentiated cell model, MucilAir, indicating that the ALI RPMI 2650 cell model is a suitable model to be used as a predictive *in vitro* model for studying IN drug permeation (Sibinovska et al., 2019).

3.1.3 | Commercially available alternative cell-type models

Commercially available alternative cell-type models used in drug permeation studies to predict nasal drug delivery are summarized in Table 3 in terms of the cell line, applications, culture conditions, growth media, and integrity.

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The pharmacological characterization of the MucilAir cell line focused on the morphology, phenotype, and expression of ATP-binding cassette (ABC) efflux transporters. The MucilAir cells form a polarized, sealed barrier, because of the homogeneous expression of adherens and tight junctions across the epithelial apical surface. The epithelial layer consisted of pseudo-stratified epithelial cells, interspersed with differentiated ciliated, basal, and goblet cells. P-glycoprotein (P-gp), BCRP, MRP1, and MRP2 were found to be the ABC transporters expressed in the MucilAir cell model, although they have been expressed at different levels. The functional activities of P-gp and BCRP were established by bidirectional permeation studies, indicating that the transporters were capable of actively effluxing the respective substrates. These attributes showed that the MucilAir cell line represents an effective tool for investigating the interaction between IN administered drugs and ABC transporters (Mercier et al., 2019).

Berger et al. (2020) cultured EpiAirway EPI-606-X in vertical diffusion chambers, in order to study the permeation of fluticasone when administered in different nasal spray formulations. The different fluticasone formulations were applied dropwise to the apical surface of the EpiAirway tissue. The fluticasone formulation containing azelastine ensured that the permeation of fluticasone occurred faster than the fluticasone-only formulation, although the total accumulated fluticasone was similar between the different formulations. This indicated that the EpiAirway cell model can sufficiently distinguish between the permeation from different formulations.

3.2 | *Ex vivo* tissue models for determining intranasal systemic and nose-to-brain drug delivery

Ex vivo models have several advantages, such as high tissue availability because it can be obtained from abattoirs where slaughtering of animals for meat production purposes occurs routinely and numerous tissue samples can be removed from one subject (Agu & Ugwoke, 2008). Furthermore, this model is simple, time- and cost-effective, and provides reproducible results (Berben, Bauer-Brandl, et al., 2018). The suitability and potential of clinical application of excised tissue permeation models has been previously validated (Schmidt et al., 2000; Wadell et al., 1999); however, it has disadvantages such as a relatively brief tissue viability window and interindividual differences occur such as age, diet, and pathology, which can potentially affect tissue morphology that can contribute to variability (Westerhout et al., 2014).

Leichner et al. (2019) determined that self-emulsifying drug delivery systems (SEDDS) provide a promising approach to nasal drug delivery following evaluation of permeability, tissue toxicity, and irritation potential of dimenhydrinate containing SEDDS across excised bovine nasal tissue. Haroon et al. (2021) conducted permeation and ciliotoxicity studies on *Centella* complexed with thiolated chitosan across caprine nasal tissue; the formulation showed good permeation of 89.44 ± 1.75% (mean ± SD, $n = 3$) of the active ingredient at 8 h, but there were signs of nasal ciliotoxicity. Evaluation of the transport of quercetin-hydroxypropyl-β-cyclodextrin inclusion complex across excised leporine nasal mucosa revealed increased permeation (0.03 ± 0.01–0.22 ± 0.05 μg/cm^2) of quercitin compared to the negligible amount transported of pure quercetin (Papakyriakopoulou et al., 2021). However, de Souza Von Zuben et al. (2021) evaluated the effect of both encapsulation and functionalization with cell-penetrating peptides on insulin release and permeation across the porcine nasal mucosa; it was determined that the addition of cell-penetrating peptides to the formulations decreased permeation.

Table 4 gives a summary of selective *ex vivo* nasal permeation, cytotoxicity, mucoadhesion, and expression analysis studies and their implemented experimental conditions.

4 | COMPARISON OF DIFFERENT MODELS USED TO STUDY INTRANASAL PERMEATION AND *IN VITRO* TO *IN VIVO* EXTRAPOLATION

Several studies have compared the permeation of a variety of compounds across different *in vitro* and *ex vivo* permeation models with their respective bioavailability in *in vivo* models, such as rats and mice. Some of these compounds studied, included different corticosteroids, atenolol, antipyrine, propranolol, quinidine, lacosamide, levetiracetam, sulfasalazine, and zonisamide (Furubayashi et al., 2020; Gonçalves et al., 2021).

Wadell et al. (2003) found that some correlation existed between the apparent permeability of passively transported drugs across excised porcine mucosa with that from nasal absorption studies performed in humans. On the other hand, drugs that make use of active permeation mechanisms such as carrier-mediated transport or efflux exhibited a less defined correlation. The authors suggested additional studies with more compounds to fully determine the influence of different transport mechanisms on the correlation coefficient.

Calu-3 cell layers have been determined to be a useful tool for the prediction and evaluation of nasal mucosal permeation in rats. This is supported by the linear correlation between *in vitro* Calu-3 cell permeability and *in vivo* bioavailability of specific drugs (such as antipyrine, acyclovir, caffeine, labetalol, norfloxacin, and ganciclovir) following IN administration in rats and the ability to quantitatively determine drug permeation from *in vitro* drug permeability values (Inoue et al., 2020).

The permeation of several selected compounds across five different cell lines used to study IN permeation was compared with the *in vivo* fractional absorption (FA) after IN administration to mice, as shown in Figure 2 (Furubayashi et al., 2020). The cell lines investigated were, EpiAirway, MucilAir, Caco-2, Calu-3, and MDCK, which are popular *in vitro* models to investigate barrier characteristics. The best correlation with the FA in rats was seen for the permeation across the MDCK cell layers ($r = 0.949$). The second highest correlation was seen in Calu-3 cell layers ($r = 0.898$). MucilAir ($r = 0.750$) and Caco-2 ($r = 0.787$) showed lower, but still relatively good, correlations with the FA in rats. The EpiAirway cell line ($r = 0.550$)

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TABLE 4 Summary of selective permeation studies conducted on excised tissue (*ex vivo*) models from different animal species.

Animal species	Nasal region	Implementation; IN/N2B, permeation or toxicity	Diffusion model	Experimental conditions						Reference
				ALI LLI	Diffusion media	Tempera-ture	pH	Duration of study	Tissue viability test	
Bovine	Nasal mucosa	IN: Permeation and toxicity	Ussing-type diffusion cells	LLI	KRB	37°C	7.4	4 h	LDH assay, Resazurin assay	Leichner et al., 2019
Bovine	Respiratory and olfactory mucosa	IN: Permeation	Navicyte vertical diffusion cells	LLI	5% (w/v) glucose	37°C	Isotonic	60 min	5% w/v glucose solution	Albarki & Donovan, 2020
Caprine	Nasal mucosa	N2B: Permeation and ciliotoxicity	Keshary-Chien permeation cells	LLI	SNF	37°C	6.4 then 5.5	8 h	Ciliotoxicity	Haroon et al., 2021
Caprine	Nasal mucosa	IN: Permeability and toxicity (biocompatibility)	Modified Franz-type diffusion cells	ALI	PBS	37°C	6.8	6 h	Histology	Thakkar et al., 2021
Human	Nasal turbinate epithelium	Expression analysis of human solute carrier; peptide transporters, OAT, OCT-mediated influx	Vertical Ussing diffusion cells	LLI	KRB	37°C	7.4 then 6.0	120 min	TEER	Dolberg & Reichl, 2018
Human	Inflamed sinus tissue: Ethmoid cavity, superior turbinate, and middle turbinate mucosa	IN: Permeation	Placed in sterile 12-well tissue culture plates	ALI	Human nasal epithelial cell media	37°C	N.R.	3 h	N.R.	Falconer et al., 2018
Leporine	Nasal mucosa	N2B: Permeation	Vertical Franz-type diffusion cells	ALI	PBS	37	7.4	240 min	N.R.	Giuliani et al., 2018

(Continues)

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TABLE 4 (Continued)

Animal species	Nasal region	Implementation; IN/N2B, permeation or toxicity	Diffusion model	Experimental conditions						Reference
				ALI LLI	Diffusion media	Tempera-ture	pH	Duration of study	Tissue viability test	
Leporine	Epithelial barrier and the connective tissue of the nasal septum	N2B: Permeation	Franz diffusion cells	ALI	PBS	37°C	7.4	120 min	The donor compartment was filled with saline solution, checking that no liquid passed to the empty receptor due to inappropriate mounting or lack of tissue integrity	Papakyriakopoulou et al., 2021
Ovine	Nasal mucosa from septum	IN: Permeation and ciliotoxicity	Unspecified diffusion cells in water bath shaker	LLI	PBS	34 ± 1°C	6.4	24 h	Histology	Abdulla et al., 2021
Ovine	Nasal respiratory mucosa	IN: Permeation and toxicity	Sweetana-Grass diffusion cells	LLI	KRB	34°C	N.R.	3 h	LY, histology, TEER	Gerber et al., 2022
Porcine	Concha nasalis media tissue	IN: Permeation	Upside-down in a side-by-side set-up; self-made system consisting of two 1.5 micro reaction tubes	ALI	DMEM:F12 supplemented with 20 U penicillin, 20 μg streptomycin and 300 I.U./mg gentamicin sulphate	35°C	6.5	5 h	N.R.	Ladel et al., 2020
Porcine	Nasal mucosa from septum	IN: Permeation	Franz diffusion cells	ALI	PBS	32°C	7.4	24 h	N.R.	de Souza Von Zuben et al., 2021

Abbreviations: ALI, air–liquid interface; DMEM:F12, Dulbecco's modified Eagle's medium/Nutrient Mixture F:12; IN, intranasal; KRB, Krebs Ringer buffer; LDH, lactate dehydrogenase; LLI, Liquid–liquid interface; LY, Lucifer yellow; N2B, nose to brain; N.R., not reported; OAT, organic anion trasnporter; OCT, organic cation transporter; PBS, phosphate buffered saline; SNF, simulated nasal fluid; TEER, trans epithelial electrical resistance.

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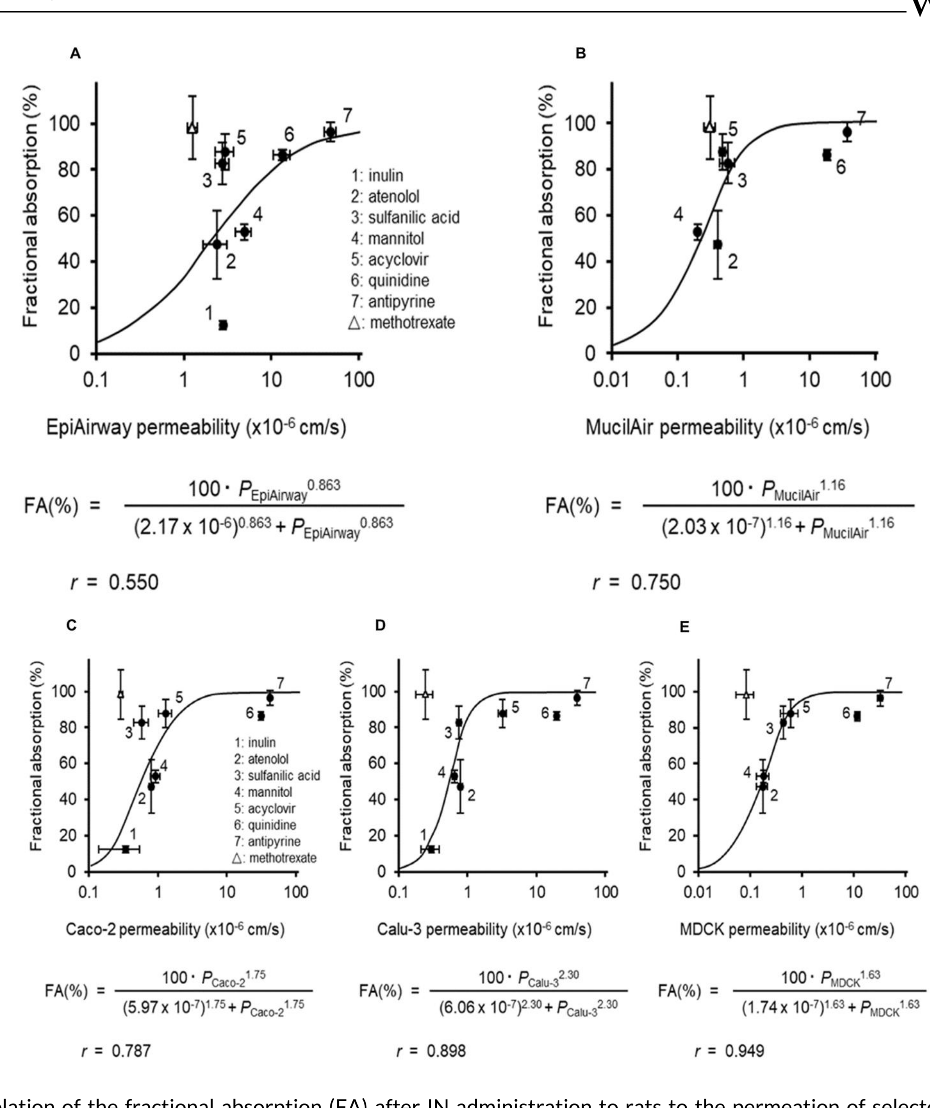

FIGURE 2 Correlation of the fractional absorption (FA) after IN administration to rats to the permeation of selected compounds across various cell lines, that is, (a) EpiAirway™, (b) MucilAir™, (c) Caco-2, (d) Calu-3, and (e) MDCK. Compounds: (1) inulin, (2) atenolol, (3) sulfanilic acid, (4) mannitol, (5) acyclovir, (6) quinidine, (7) antipyrine, (Δ) methotrexate. FA: fractional absorption. Methotrexate data are plotted as an open triangle (Δ). The data are expressed as the mean ± S.E ($n = 3$ or 4). These graphs are reproduced with permission from Furubayashi et al. (2020). IN, intranasal; MDCK, Madin-Darby canine kidney.

showed the poorest correlation between permeability and FA. This poor correlation could possibly be ascribed to the leakier tight junctions that is formed by the EpiAirway cell layers, and thereby possibly overestimating paracellular permeation.

Esim et al. (2020) compared the IN delivery of eletriptan hydrobromide loaded polylactide-co-glycolide nanoparticles into the brain to that after IV administration in Wistar rats. A higher total amount of drug reached the brain upon IN administration, with an AUC_{brain} double that of an IV administration.

When studying the IN delivery of various pain medications, Touitou et al. (2021) found that the IN administration of soft nanovesicle drug formulations increased the concentration of tramadol in the plasma and brain, 2–5 times higher than that of the oral and nasal nonvesicular formulation controls. Another drug used for pain management, ketoprofen, showed a three times higher maximum concentration when administered as a soft nanovesicle, compared to the oral and nasal controls.

Gerber et al. (2022) found that both RPMI 2650 cell line models and excised ovine respiratory tissue presented with similar permeation for a large molecular weight compound (fluorescein isothiocyanate [FITC]-dextran 4000 Da) and an efflux transporter substrate (Rhodamine 123). Similarities were also found with regard to immunohistochemistry properties, tight junction expression, and epithelial thickness.

5 | EXPERIMENTAL CONSIDERATIONS DURING DRUG PERMEATION STUDIES TO DETERMINE INTRANASAL DRUG DELIVERY

In order to draw a correlation between *in vitro* or *ex vivo* and *in vivo* results, and ultimately ensure that the model is of value to the drug development process, researchers should develop and adhere to standardized procedures with clear working parameters and

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consequently publish thoroughly and accurately reported methods. The factors influencing the outcome and value of experiments and the collected data can be classified as preexperimental, experimental, and postexperimental factors (Cabrera-Pérez et al., 2016; Volpe, 2010).

Preexperimental factors such as the source and process of procurement of biological materials or cells, preparation of tissue samples or cell lines, maintenance and evaluation of integrity, and viability can have major implications on the permeability data obtained from the selected model (Volpe, 2010). Preparation methods such as delipidation of tissue samples with organic solvents (e.g., chloroform, methanol) are employed in order to avoid ultraviolet absorbance interference during analysis (Naguib et al., 2020). However, this could notably alter lipoid organization and loss of barrier function, which could have drastic influences on the data obtained from the permeation study (Ponec, 1992). The use of frozen excised tissue for permeation studies should be avoided, as freezing the specimens could result in the formation of artifacts (Nicolazzo et al., 2003). Although preferred, it is not always possible to obtain fresh tissue. Adding cryoprotectants to tissue prior to freezing delivers variable results (Marxen et al., 2016). Therefore, it has been proposed that adequate comparison tests be performed on both fresh and frozen tissue models prior to substitution to determine the suitability of the method on the specific tissue and drug investigated (Nicolazzo et al., 2003).

Experimental factors such as medium composition, the presence of medium in both chambers (ALI or LLI), the pH of the medium in both chambers, the method for system agitation (bubbling or magnetic stirring), plastic support material type, solute concentration, volume of solute administered, and temperature have the potential to affect epithelial barrier properties (i.e. integrity, permeability, and transporter expression) and the thickness of the unstirred water layer (Cabrera-Pérez et al., 2016). In addition, specifications such as the contact area of the epithelial layer to the solute and the sampling schedule; only acceptor chamber or both, number of samples, volume of sample withdrawn, and medium replacement for the maintenance of sink conditions determine the calculation methods to be implemented following sample analysis.

Postexperimental factors refer to the analytical and mathematical methods and assumptions used to elucidate the mechanisms as expressed by the obtained data (Cabrera-Pérez et al., 2016).

5.1 | Effect of the region from which the epithelial tissue is sourced in the nasal cavity

When sourcing nasal tissue for *ex vivo* permeation studies, the regional site in the nasal cavity from which it is excised should be taken into consideration. A study conducted by Fransén et al. (2007) on excised porcine nasal epithelium found that the permeation of dihydroergotamine was significantly higher across excised olfactory epithelium (permeation coefficient [P_{app}] = 5.04×10^{-7} cm/s) than across excised respiratory epithelium ($P_{app} = 1.48 \times 10^{-7}$ cm/s). It was proposed that the increased permeation across olfactory epithelium can be attributed to the facilitated transport within the olfactory nerves in combination with other permeation mechanisms. Koushik and Kompella (2010) reported a regional variation in the transport of deslorelin across bovine nasal epithelium collected from the anterior medium turbinate (0.2 $\pm$ 0.06%), posterior medium turbinate (1.6 $\pm$ 0.1%), and the posterior inferior turbinate (2.85 $\pm$ 0.3%) regions. They ascribed the variation in permeation potentially to differences in the expression of transporters in the different tissues. However, there are limited experimental data available on transporter expression and its effect on drug transport in the different regions of the human nasal mucosa. The clinical significance of specific drug substrates can be determined by the identification of transporters, with a major role in drug transport within the nasal route through gene expression analysis (Moh'd Al-Ghabeish, 2014). Furthermore, substrate specificity can differ between two tissue types within the same animal species; the expression of enzymes similar to those found in hepatic tissue can present with different specificity within nasal tissue. Variation in enzymatic and transporter expression can also be expected due to pathology, age, gender, and other lifestyle factors (Oliveira et al., 2016). A recent study on the expression and functional activity of four solute-linked carriers (two organic cation transporters and two organic anion transporters) in the RPMI 2650 cell line and nasal models was conducted by Dolberg and Reichl (2018). The study demonstrated that, despite minor differences in functional activity, there is good correlation between RPMI 2650 cells and explants of human nasal mucosa regarding their solute carrier expression profiles.

Du et al. (2006) investigated the permeation of nalmefene hydrochloride across different regions of ovine nasal mucosa and found that P_{app} values were similar across middle turbinate, posterior septum, and superior turbinate, while the anterior septum and inferior conchae exhibited lower P_{app} values. They ascribed these findings to thickness and morphology of the epithelium in the respective regions. The thickness of the mucosa from different regions were ranked as follows: inferior turbinate mucosa (1500–2000 μm) > anterior septum mucosa (1200–1700 μm) > superior turbinate (900–1300 μm) > posterior septum mucosa (500–600 μm) > middle turbinate (450–600 μm). Therefore, the region from which the mucosa is excised should be considered and meticulously noted in all *ex vivo* permeation studies.

From an anatomical and physiological perspective, it might be recommended to use respiratory tissue to represent systemic delivery and olfactory tissue to represent direct N2B delivery (Fransén et al., 2007; Wadell et al., 2003). At this time, there is insufficient data to prove significant differences in drug permeation across the different regions of the nasal cavity. In general, it is recommended that the epithelial tissue be used where drug absorption will occur, namely, respiratory tissue for systemic drug delivery and olfactory tissue for N2B drug delivery. It is, however, important to always disclose the defined sampling region from which the tissue was obtained for the *ex vivo* studies for the purpose of comparison.

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5.2 | Diffusion chamber devices for *ex vivo* permeation studies

Side-by-side or horizontal diffusion chamber devices are routinely used to perform *ex vivo* permeation studies (Bartos et al., 2021). One of the first side-by-side diffusion cell devices for membrane permeation measurements was described by Ussing and Zerahn (1951) and later this apparatus was modified by Grass and Sweetana (1988). For the Ussing type diffusion apparatus, an excised epithelial membrane is clamped between two acrylic chambers, which are filled with preheated diffusion medium and carbogen gas (a mixture of 95% O_2 and 5% CO_2) is continuously bubbled through the medium to maintain tissue viability and ensure good hydrodynamics. The drug substance is added to the donor chamber compartment whereafter the amount of drug accumulated in the acceptor chamber is measured as a function of time (Grass & Sweetana, 1988). A possible limitation to this apparatus is the occurrence of adhesion of drug molecules to the surfaces of diffusion cells (Agu & Ugwoke, 2008; Osth et al., 2002).

The Franz diffusion cell device is another widely implemented diffusion apparatus for membrane permeation studies. The apparatus is set up vertically with an upper donor chamber, which is attached to a flat ground glass joint that serves as the acceptor chamber. A biological membrane (e.g., excised epithelial tissue) is placed horizontally between the two chambers through which the drug can permeate. The acceptor chamber contains diffusion medium and is surrounded by a water jacket that circulates heated water to maintain and simulate body temperature within the chamber. The acceptor chamber is fitted with a sampling port for the withdrawal of samples. The medium in the acceptor chamber is constantly agitated by a magnetic stirring bar (Mattiasson, 2020).

Bartos et al. (2021) compared the suitability of a vertical diffusion cell apparatus (Franz diffusion cell device) to a horizontal diffusion apparatus (side-by-side type diffusion device) for different formulations (including a spray, gel, and powder). The study found that spray and powder formulations could be investigated by horizontal diffusion devices; however, the investigation of gel formulations is not desirable in this type of diffusion device. Implementation of vertical diffusion cells delivered a more uniform distribution of the gel formulation on the mounted membrane. Based on these findings, the authors recommended the application of horizontal diffusion cells for liquid and solid nasal preparations, while vertical diffusion cells should be used for semisolid formulations.

Osth et al. (2002) found that when an ALI is created in vertical diffusion cells where diffusion medium is only applied to the acceptor chamber, it did not affect the TEER or P_{app} values as compared to that of an LLI in horizontal diffusion cells. The results from their transport study also indicated that both devices could be implemented to investigate both transcellular and paracellular transport. The relative position of the chambers and diffusion membranes implied that gravitational forces may have an effect on the permeation of molecules from a formulation across the membrane in the case of vertical diffusion cells, while it may affect the distribution of the formulation in the chambers of the horizontal diffusion cells. A limitation to vertical diffusion cells is that the donor chambers cannot be agitated to mimic ciliary movement of the nasal cavity or ensure uniform drug distribution in the donor chamber.

5.3 | Viability and integrity of cell monolayers and excised tissues in *in vitro* studies

There are several tests that can be conducted to confirm the viability and integrity of excised tissues; often, more than one test should be considered to prevent false results when conducting experiments using excised tissues and cell monolayers (Schmidt et al., 1998). All *ex vivo* permeation studies should be conducted within the window of tissue viability (3–3.5 h) by taking care to collect the tissue directly after animal sacrifice and process the tissue as quickly as possible (Du et al., 2006; Wheatley et al., 1988). Bubbling carbogen gas through the permeation medium will contribute to maintaining the tissue integrity (Agu & Ugwoke, 2008). The selection and use of an appropriate diffusion medium with a physiologically compatible composition that can facilitate and maintain cell biochemical processes is essential to assist in maintaining tissue viability and integrity (Albarki & Donovan, 2020; Maitani et al., 1997). Exogenous glucose has, for example, been found to influence the electrophysiological resistance of nasal mucosa. The absence of glucose caused a notable reduction in the TEER of the mucosa within 30 min (Schmidt et al., 1998).

Different methods can be employed to monitor the integrity of biological membranes used by *in vitro* and *ex vivo* models such as electrophysiological measurements (i.e. TEER measurements) and measurement of the permeability of exclusion marker compounds such as Lucifer yellow (Dimova et al., 2005).

TEER is the real-time electrophysiological measurement based on resistance to the conduction of an electrical current across epithelial membranes (Srinivasan et al., 2015). This test can be implemented to evaluate the integrity of a membrane prior to a permeation study to indicate mechanistic damage inflicted during excision or mounting. This measurement can also be implemented throughout and after conducting a permeation study to determine if the tissue viability was maintained or to determine if the drug or other components may have had a detrimental effect on the membrane. It is recommended that TEER measurements are implemented in combination with an additional membrane integrity test (Sutton et al., 1992), such as the permeation of marker molecules (e.g., LY).

In order to confirm the integrity of excised nasal epithelium tissue and cultured cell monolayers for permeation studies, the P_{app} of marker molecules can be determined (Dimova et al., 2005; Sousa & Castro, 2016). Transcellular lipophilic compounds that are commonly used as marker molecules include propranolol and dexamethasone. The paracellular hydrophilic compounds commonly used are LY, sodium-fluorescein (Flu-Na), mannitol, sulforhodamine, atenolol, sucrose, and albuterol (Dimova et al., 2005; Pozzoli et al., 2016; Sharma et al., 2019; Sibinovska et al., 2019; Sousa & Castro, 2016). The

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permeability of high molecular weight compounds can also be used to investigate monolayer integrity and the most used compound is FITC dextran (MW ≥ 3000 Da) and polyethylene glycol 4000 (Sibinovska et al., 2019; Wengst & Reichl, 2010).

A histology assay can evaluate the effect of mechanistic tissue damage that could potentially be inflicted during the excision and mounting of the epithelial tissue and/or to determine the potentially toxic effect of a drug or excipient on the tissue (Agu & Ugwoke, 2008). Specimens are taken prior to and after conducting permeation studies, whereafter the tissue samples are fixed in normal-buffered formalin (10%), dehydrated, embedded in wax, sliced with a microtome, rehydrated, stained, dehydrated, and evaluated under a microscope for physiological, anatomical and morphological features (Gerber et al., 2022).

When establishing new permeation methods or models, a validation should be performed to confirm suitability. It is suggested that the guidelines set out by the European Medicines Agency for evaluation of Caco-2 cell assay suitability (ICH, 2020) be extrapolated to nasal permeation models; the experimental permeability values and the extent of drug absorption of known zero, low (<50%), moderate (50%–84%), and high (≥85%) permeability model drugs should be determined. Additionally, integrity should be confirmed by zero permeability compounds and comparing TEER measurements, prior to and after an experiment. All experimental considerations of the study method should be described in reasonable detail; sampling species and region, preexperimental conditions (i.e. cold buffer, frozen tissue samples, time taken to mount in chosen diffusion chamber setup, etc.), incubation conditions, permeation media, drug concentrations applied, sampling conditions (i.e. sink conditions, uni- or bidirectional transport, time intervals of sampling, etc.), analytical methods, and equations used to calculate permeability (such as P_{app} or flux).

6 | CONCLUSION

An increased interest in IN and N2B drug delivery has led to an increase in the variety and availability of different *in vitro* nasal models available. These *in vitro* nasal models are used to study the permeation and metabolic characteristics of nasal mucosa, by using different cell culture models and excised nasal tissues (Erdő et al., 2018).

Nasal cell culture models are promising tools to determine transport mechanisms and to test novel approaches to enhance drug absorption and transport. To study nasal anatomy, primary cell cultures were developed by isolating nasal epithelial cells from a variety of species, including human, rat, pig, and rabbit. There are many advantages associated with using *in vitro* cell culture models, like the ability to determine drug transport pathways and mechanisms, the ability to investigate drug permeability rapidly and effectively, the capability to control the growth conditions to study epithelial cell growth and differentiation, and the ability to minimize and limit the use of expensive (and controversial) animal subjects (Schmidt et al., 1998). The main limitation in using primary cell cultures is the difficulty in obtaining reliable and sufficient tissue sources, especially if human tissue is preferred. From primary cells, different cell lines were created with an extended or permanent lifespan in order to overcome the shortage of human nasal tissue and limited isolation of nasal cells from individual donors (Schmidt et al., 1998). Although cell monolayer models present with functional properties similar to human epithelium, the use of this *in vitro* model is restricted by the lengthy duration of cell growth cycles, high monetary cost, risk of microbial contamination, and both intra- and interlaboratory variability (Corti et al., 2006; Volpe, 2010).

Ex vivo nasal models have the potential to provide valuable information on the permeability, metabolism, efflux, and toxicity of molecules across the nasal epithelium and the strategies implemented to improve drug absorption (Erdő et al., 2018). This model can be utilized to determine the effect and concentration dependency of enhancers on flux, the reversibility and recovery time of the enhancement, the level of tissue damage, the respective mechanisms involved, and the effect on ciliary beat frequency (Agu & Ugwoke, 2008). *Ex vivo* models have several advantages, such as high tissue availability as compared to whole animal or cell culture models, because it is obtained from abattoirs where slaughtering of animals for meat production purposes occurs routinely and surmounting tedious ethical issues such as nonessential animal sacrifice. An added ethical advantage is that fewer subjects are necessary, as numerous tissue samples can be removed from one subject. This in turn also decreases intraday variability between studies, as tissues can be screened to ensure quality selection. The system is easily monitored, which allows relatively low labor to ensure tissue viability. The model provides the opportunity to evaluate and potentially improve on a variety of mechanistic factors such as permeation and mucoadhesion, metabolism, efflux, and toxicity. This model can deliver quantifiable results regarding passive diffusion and aid in the identification or characterization of transport routes (Agu & Ugwoke, 2008). It is important to note the limitations to the *ex vivo* model in order to make an informed decision prior to employing the model. The thickness of the epithelial tissue varies widely per animal, as well as within the nasal cavity. Further variety is caused by the amount of connective tissue and mucus left on the sample after preparation (Erdő et al., 2018). This variability in epithelial thickness and the lack of standardization of experimental conditions complicates the extrapolation and comparison of data obtained from *ex vivo* studies to one another and to *in vitro* or *in vivo* studies (Agu & Ugwoke, 2008).

Selection of a particular species is generally based on availability and access to tissue rather than the correlation of the species anatomical and cellular morphology to that of humans. The rationale being that the focus of permeation studies is often mainly on the investigation or evaluation of the implemented formulation strategy rather than *in vitro*–*in vivo* correlation (Agu & Ugwoke, 2008). The use of *in vitro* and *ex vivo* experimental models also has the limitation that it does not reflect pathophysiological conditions; thus, it cannot accurately predict the effect of a disease on drug absorption. Regarding monetary cost, *ex vivo* tissue systems are thus considered

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a more attractive method for permeation testing. This model is beneficial, as it is simple, time- and cost-effective, and provides reproducible results (Berben, Bauer-Brandl, et al., 2018). However, a relatively brief tissue viability window and interindividual differences such as age, diet, and pathology can potentially affect tissue morphology that can contribute to variability (Westerhout et al., 2014), subsequently reducing this model to a low-to-moderate throughput system (Berben, Brouwers, et al., 2018).

There are still numerous knowledge gaps within the field of IN drug delivery. For example, a comprehensive region-specific morphological comparison and drug permeation evaluation needs to be conducted on *ex vivo* models to ensure the appropriate selection of epithelial tissue in the future for standardization. Furthermore, existing and new experimental models should be validated in full with the use of reference drugs, TEER, and histological/morphological evaluation. There is also a dire need for the proper evaluation and extrapolation of permeation data between *in vitro* and *ex vivo* to *in vivo* models. With the acquirement of this knowledge, the full potential of N2B delivery can be predicted for drugs on a high-throughput scale.

It can therefore be concluded that the consideration of *in vitro* cell-based or *ex vivo* tissue models be implemented according to the relevant needs and capabilities of the project, researcher, and interlaboratory.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article, as no datasets were generated or analyzed for this review article.

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