HAL
open science

In vitro – in vivo correlation of intranasal drug deposition

S. Le Guellec, S. Ehrmann, L. Vecellio

To cite this version:

S. Le Guellec, S. Ehrmann, L. Vecellio. In vitro – in vivo correlation of intranasal drug deposition. Advanced Drug Delivery Reviews, 2021, 170, pp.340-352. 10.1016/j.addr.2020.09.002 . hal-03518808

HAL Id: hal-03518808

https://hal.science/hal-03518808

Submitted on 3 Jun 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

In vitro – *in vivo* correlation of intranasal drug deposition

S. Le Guellec (1,2), S. Ehrmann (1,3), L. Vecellio (1)

Affiliations :

1. INSERM, Centre d'étude des pathologies respiratoires, U1100, Tours, France ; and Université de Tours, Tours, France
2. DTF-Aerodrug, Tours, France
3. CHRU Tours, Médecine Intensive Réanimation, CIC INSERM 1415, CRICS-TriggerSep network, Tours, France

Corresponding author: Laurent VECELLIO, INSERM, Faculté de Médecine, Centre d'étude des pathologies respiratoires, Université de Tours, 10 Bd Tonnellé, 37032 Tours cedex, France

vecellio@med.univ-tours.fr

Abstract

In vitro – *in vivo* correlation (IVIVC) allows prediction of *in vivo* drug deposition from a nasally inhaled drug based on *in vitro* drug measurements. *In vitro* measurements include physical particle characterization and, more recently, deposition studies using anatomical models. Currently, there is a lack of IVIVC for deposition measurements in anatomical models, especially for deposition patterns in various nasal cavity regions. Therefore, improvement of *in vitro* and *in vivo* measurement methods and knowledge about nasal deposition mechanisms should help IVIVC in the future.

Graphical abstract: *In vitro* parameters to be considered to predict *in vivo* nasal drug deposition: particle characteristics generated by drug delivery devices, patient ventilation and anatomy using anatomical model tools, for example.

Keywords: nasal spray; deposition; cast; nebulizer; *in vitro*; *in vivo*; particle size; anatomical model; devices

1. Introduction

Aerosol inhalation has been studied for many years in the fields of pollution and pharmaceutics. The main focus has been on lung deposition, to prevent lung contamination by pollutants or to administer drugs by inhalation to treat patients. The nose has generally been considered as a whole in terms of anatomy and described as a "filter." However, the nasal cavity has a complex structure, with specific anatomical regions to be targeted by drug delivery (Figure 1). It is a large, air-filled space above and behind the facial visible nose, corresponding to a surface area of about 160 cm^2 and volume of 15 mL. The nasal septum divides the cavity into two fossae, with each fossa extending from the vestibule of the nose to the nasopharynx. Behind the vestibule and along each outer wall, there are three turbinates (superior, middle and inferior), which theoretically serve as anatomical targets for locally acting corticosteroid and antibiotics [1], as well as drugs intended for systemic effects [2]. Beside and above the superior turbinate is the olfactory region of the nasal cavity, with the surface area corresponding to 5 cm^2 [3], which may be targeted for *nose to brain* drug delivery [4]. The frontal, maxillary and anterior ethmoidal sinuses open into the middle meatus, which represents the regions of interest (ROIs) for sinus targeting [1]. The upper part of the rhinopharynx represents the main lymphatic drainage region of the nose, making it an attractive target for vaccine delivery [3].

Among nasal drug delivery, there is an increasing interest to deliver drugs targeting these specific anatomical regions. However, to date, there is no clear relationship between the anatomical site of drug deposition and clinical efficacy. Beyond targeting the nose, itself, nasal drug delivery can serve for targeting the brain, the immune system (*e.g.*, vaccines) or other systemic effects. From a physiological point of view, the nose is a non-vital organ, and thus, presents advantages in terms of drug toxicity risk by comparison to the lung. Conversely, nasal drug deposition is not always desired and one aims to avoid it when targeting the lung. For example, aerosol administration via nasal cannula during nasal high flow therapy is a new way for drug administration, potentially well tolerated, but exposing to significant nasal deposition [5]. Therefore, deposition studies in the nose may have a double interest: either with the aim of favoring nasal deposition or with the aim of avoiding it when targeting the lung.

Devices used for nasal drug delivery generate particles from a few micrometers (nebulizers) to hundreds of micrometers (spray pumps) with a large range of particle velocity. The range of particle characteristics is much larger than for traditional lung aerosol devices, generating particles between 1-5 µm. Drugs can be delivered through the nostrils or during the expiratory phase through exhaled aerosol deposition [6]. Taken together, there are many differences between lung and nasal drug delivery in terms of aerosol characteristics, anatomy, and mechanism of particle deposition, and thus, *in vitro – in vivo* correlation needs distinct specific considerations.

From a scientific point of view, *in vitro* studies are conducted before clinical trials in order to optimize drug delivery methods or to demonstrate the *in vitro* targeting. The *in vitro* step is based on the hypothesis of a positive correlation between *in vitro* measurement and *in vivo* deposition.

In this review, we will present the tools available to comprehensively characterize particles and their nasal deposition using *in vitro* and *in vivo* methods and the correlation between both. Moreover, we will examine the unmet needs to improve the *in vitro – in vivo correlation* (IVIVC) in nasal deposition.

2. *In vitro* methods

2.1 Aerosol physical characteristics measurements

2.1.1. Regulatory methods

Nasal spray pump performance can be evaluated in accordance with different regulations, such as those set out by the EMA, US FDA [7, 8] or one of the pharmacopoeias [9]. Physical characteristics of the spray are then measured with precise methods. Shot weight measurements consist of weighing single actuations and provide information about the consistency of the dose delivered to the patient; however, it does not measure the delivered active compounds per dose. The delivery dose consists of spray actuation in a suitable container to collect the drug and to assay it in order to determine the mass of active compound delivered per dose.

Droplet size distribution (DSD) is measured by laser diffraction at two distances from the actuator orifice. The spray generation over time can be broken down in three phases: flow formation, developed flow and flow dissipation [10]. The DSD is measured *in vitro* during the fully developed phase; however, patients receive the drug during the three phases. Results are generally reported in terms of D10, D50, D90, span and percentage of particles smaller than 10 µm to predict lung penetration. In addition, laser diffraction does not measure the drug contained in the droplets, nor aerodynamic diameter. In the case of a liquid solution, the particle size distribution should be similar in terms of the volume and drug mass but it could be different for a liquid suspension or a powder including active compounds and excipients.

The cascade impactor is the measurement device allowing drug quantification within the particles and measurement of the aerodynamic diameter. It has been initially designed for measurements of aerosols and not for spray delivery. Regarding nasal systems, particle size is measured by a cascade impactor using a glass expansion chamber and a pre-separator. Preferably, the glass expansion chamber would have a volume larger than 2L [11]; however, its relevance is questionable regarding its volume and its geometry compared to nasal cavities [12]. Moreover, it is not able to measure the complete distribution of the spray due to its size range limitation, which is smaller than the spray DSD. Rather, cascade impactor

testing is intended to quantify the mass of the drug in small droplets to evaluate the dose that could reach the lungs.

Evaluation of spray pattern and plume geometry consists in shape/geometry measurements of the spray. It uses imaging methods to determine the angle and the width of the spray plume, the ovality ratio and the area of the section of the spray. Measurements are generally performed at two different distances from the actuator. Moreover, spray pattern evaluation is very challenging due to a high measurement variability, which can be explained by the differences in terms of the testing method itself, including time lag between consecutive actuations, day-to-day variability and laboratory-specific variability [13]. These measurements do not take into account the amount of drug contained in the different parts of the spray, which may be heterogeneous.

Spray pattern and spray plume geometry measurements are generally performed using automatic mechanical actuators in order to control the stroke length, actuation velocity and actuation force and thus, favor reproducibility of the spray characteristics [14, 15], even if it does not correspond to patient variability observed in clinical practice [16-18].

For a suspension in a nasal spray, drug particle size of the active compound has the potential to influence the drug bioavailability to the anatomical target zone [10]. In this context, Morphologically-Directed Raman Spectroscopy (MDRS) has emerged as a new method able to characterize the particle size and the shape of active pharmaceutical ingredient (API) in the drug product. Although MDRS has been recommended for the approval of generic mometasone furoate nasal suspension spray [19], it measures the API particle size in the total collected spray and not in the different droplets sizes produced by the spray, which could impact the API deposition distribution in the nasal cavity and, consequently, the clinical effect.

Regarding the nasal nebulizer's performance, there is no medical device standard regarding its *in vitro* evaluation, even if EN ISO 27427 [20] could be technically applied to quantify the aerosol product (inhaled mass) and measure the particle size by cascade impaction. Although the FDA Guidance describes tests for nasal aerosol, it concerns multidose devices such as nasal pressurized metered dose inhalers, and not nebulizers.

2.1.2 Alternative methods

Alternatives to regulatory methods which involve using imaging techniques for spray pattern and plume geometry evaluation have been recently developed. They consist in a modified United States Pharmacopeia (USP) induction port to characterize nasal spray plume geometry [21]. It uses the inertial method to collect the drug along the segmented induction port reflecting the distribution of the angle of the droplets emitted from the nozzle. MMPA (mass median plume angle), is then calculated and corresponds to the angle where 50% of the drug is deposited. This method enables measuring the distribution of the active compounds in the spray angle, which cannot be done by imaging techniques.

The particle velocity is not measured by regulatory methods and could have an impact on nasal deposition. Phase-Doppler anemometry is a method allowing the simultaneous measurement of droplet velocity and size. It has been observed that nasal spray pumps generate large-sized droplets, which tend to have higher velocities than small generated droplets [22].

Aerosol characteristics are generally measured at different distances from the nozzle because the spray does not have the same characteristics at different nozzle distances. Particle image velocimetry and particle/droplet image analysis is used to measure the external spray characteristics and the DSD from a continuous spray. A study from Inthavong *et al.* [23], which involves using this technique, has observed a heterogeneous distribution of particle size and velocity in the different regions of the spray over the atomization duration. These high precision measurements could be very interesting for particle deposition prediction in situations where the spray plume cannot develop to its full size, as it may happen inside the nasal vestibule *in vivo.* Combined with the intrinsic heterogeneity of the spray this may have important consequences on deposition prediction. Indeed, particle image velocimetry has the advantage of measuring the particle size in different regions of the spray over time; however, it is not able to measure the drug contained in the droplets.

These new methods provide additional information to regulatory methods, but no single method completely characterizes the spray and enables comprehensive deposition prediction in the different regions of nasal cavities.

2.2. Anatomical models

The modelling of nasal cavities is widely used to study the intranasal deposition of nasally inhaled drugs. This method serves as an *in vitro* tool that mimics upper airway geometry more or less closely to human geometry.

2.2.1. Different types of models

Thirty-eight different anatomical models have been identified in the literature and listed in Table 1 [24-85]. The *in vivo* representativeness of these models was analyzed and is presented in Figure 2. The first models were created using glass and were only composed of a simple tube with two openings (nasal fossae) communicating with a sinus model [24, 26]. Several more recent models share the same kind of simplified geometry. Nasal fossae have been modelled by a plastic tube [27,32], bottles [33] or by using a plastic cavity with simulated turbinates [28, 29]. The nasal valve is also simulated by a narrowing in the tubes [32]. These first structures could be equipped with narrow openings and parallel cavities to simulate paranasal sinuses using glass vials or syringes [27, 29, 31].

Cadaver heads may intuitively be considered as an ideal representation of human geometry [76-80]; however, preservation issues and tissue retractions can rapidly limit the uses of such models. Plastination is an old preservative method consisting in replacing water and lipids by silicon, adapted by Durand *et al.* [81] and used on cadaver heads to overcome these limitations. Their plastinated models enable a great degree of preservation of anatomical structures and have been validated by rhinomanometry in terms of nasal resistance [82, 83]. These models obtained from cadaver specimens present a high level of complexity *in vivo* representativeness.

Other models have been designed to mimic human geometry with a higher precision. Specifically, 3D-printed nasal models have been made by stereolithography using fused plastic deposition [69] or resin polymerization [67, 70].

Since the first 3D-printed model [34], this technology has gained great interest. This modelling method allows increased sophistication and accuracy in *in vitro* geometry realism. So far, all reported models are relatively complete in terms of anatomical features (Figure 2, Table 1) [35-75]. This technique was particularly used to mimic pediatric upper airways [61, 62, 64, 65] to replace in vivo studies among children, which are required strong ethical considerations and procedures. A 3D-file of a pediatric nose and throat model is available to download for free [59], with other models being commercially available (Koken®, Japan). At the same time, bench studies services using personalized models have developed [74, 75].

2.2.2. Deposition quantification

Global nasal deposition has been investigated in *in vitro* anatomic models according to different aerosol characteristics and physical parameters [86-87], or in comparison to simulated lung deposition [62, 63]. Most studies investigated the distribution of the deposited aerosol in different parts of the nasal model [29, 47, 69]. Therefore, dismantlable models have been developed. Turbinates, maxillary sinuses and the ethmoid region are the principal regions of interest (ROIs) studied for nasal aerosol deposition. Spray deposition patterns are usually investigated in models with one or two nasal fossae geometries containing the three turbinates [37, 39, 40, 43, 50, 56] without paranasal sinuses. Moreover, some authors have studied the influence of different parameters, such as the addition of vibrations or the particle size, on the capacity of the aerosol to penetrate into the maxillary sinuses [29, 25, 84, 85, 88]. In the same way, deposition of aerosolized drugs in the ethmoid region is investigated with the aim to target the olfactory region [89] and/or to reach the brain [51]. In parallel, nasal anatomic models were also used to investigate enhanced lung deposition of inhaled aerosols [61, 63].

For aerosol deposition experiments with nebulizers, the anatomic model is usually ventilated by connecting a respiratory pump to mimic the patients' breathing [62, 69]. DSD of the aerosols inhaled through the model may also be studied by connecting the model to a cascade impactor [61]. In most nasal spray deposition studies, no ventilation is generated through the nasal model [40, 72, 90]. However, in clinical practices, different breathing recommendations are made to patients during and after nasal spray administration, such as

sniffing. However, few authors have included specific breathing patterns in their *in vitro* testing with nasal sprays [37, 41].

Anatomical models have been improved beyond geometric realism to mimic human cavities by adding artificial mucus [26], humidification or a flexible nose and face. For example, different mucus preparations were used by Sawant [54] to coat a pediatric (12-year-old) model. Coating simulates the presence of a mucosal surfactant, or mucus, plays a role in entrapping inhaled particles and allows humidification of the model [47, 91]. Humidification can also be obtained by connecting a heater/humidifier to the model [71]. Recently the possibility to print the vestibule or the face in flexible materials enables simulating aerosol administration more closely to *in vivo* conditions with an improved accuracy in interface positioning [72, 92].

Nasal deposition within the anatomic models can be measured by different methods, some of which are also used for *in vivo* clinical evaluation. Specifically, gamma scintigraphy is widely used on nasal casts. It allows radiotracer quantification for total or regional nasal deposition calculations [69, 62, 29], but has limitations for precise quantification in small ROIs [69]. Moreover, 3D single photon emission computed tomography coupled with computed tomography (SPECT/CT) enhances the accuracy of measurements [93]. The attenuation property of the model, which depends on the material and the model type, is not systematically included in studies. Tracers may also be used to visualize and/or quantify nasal deposition, by photography and/or UV-spectrophotometry dosages. In this case, the model used should be transparent or dismantlable to collect and analyze the tracer deposition [54 71, 94]. Nasal endoscopy can be used to visualize the tracer deposition in closed models. Durand et al. have inserted a humidity sensor into maxillary sinuses to detect the aerosol penetration in these cavities [81]. Recently, iodinated aerosols deposition quantification was directly performed by computed tomography (CT) [72].

3. *In vivo* methods

In vivo assessment is a core requirement for IVIVC analysis. The anatomical complexity of the nasal cavity and its high interspecies variability renders most animal *in vivo* models unsuitable to study human nasal spray deposition. Therefore, human clinical studies are required to fully validate *in vivo* the results observed on nasal casts and in other *in vitro*

experiments [63]. Given the various targets pursued through nasal drug deposition (nasal mucosa, sinuses, brain, systemic effects, etc.) and the complexity of setting up clinical studies, some authors evaluated direct or surrogate markers of biological efficacy. In such studies, the efficacy measurements may preclude concomitant nasal deposition measurement and thus, correlation to *in vitro* experiments. In fact, given the complexity of the nasal anatomy, the correlation between nasal drug deposition and some biological effects may be less straightforward than for other targets of inhaled drug delivery, such as lung deposition.

3.1. Direct nasal deposition quantification methods

A great number of studies have evaluated nasal deposition of various tracers delivered as nasal sprays to humans. The first studies used direct endoscopic visualization of nasal deposition of solutions containing a dye (methylene blue, fluorescein, etc.) sprayed in the nose of healthy subjects or patients [94, 96]. This initially qualitative technique was improved by some groups by implementing imaging post-processing techniques to quantify dye deposition. To improve quantification of deposition, a great number of studies used radiolabeled solutions coupled with scintigraphy measurement of deposition [30, 96-103]. An important limitation of such imaging techniques is related to precisely delineating the ROIs within the nose to quantify deposition. In addition, anatomical landmarks external to the nose may be radiolabeled, while the nose size can be estimated by acoustic rhinometry in order to delineate specific ROIs to quantify deposition [96, 100, 103]. To more precisely estimate regional deposition within the nose, deposition scintigraphy may be combined with anatomic imaging, such as magnetic resonance imaging (MRI) [96, 103], CT [103] or radioactive gas ventilation scintigraphy [30, 99]. Currently, SPECT/CT using various sprayed tracers represents a significant improvement from 2D planar scintigraphy [93]. Positron emission tomography (PET) may enable to envision specific drug labeling or nasal physiologic effects quantification [104]. However, the nose is a relatively small organ and regions with different functional implication are located in very close proximity; thus, deposition analysis may be impacted by variability in ROIs delineation and small patient-to-patient anatomic variation may have an important impact on the accuracy of regional deposition evaluation within the nose. Correction for local tissue attenuation is also a challenge that has a major

impact on quantitative deposition analysis, which is required to move beyond qualitative deposition patterns [105].

3.2. Indirect quantification methods

Depending on study design and objectives, nasal deposition of an inhaled drug may be quantified indirectly. Thus, studies evaluating pulmonary drug delivery through the nasal route can be used to indirectly assess nasal deposition as well. Whatever the tracer and quantification technique, the mass balance principle considers that the part of a given amount of a tracer delivered through the nose not recovered in the lung, upper airways, digestive tract or systemic circulation has been deposited in the nose. This may be considered positively if the nose was the target and negatively otherwise [100]. Systemic pharmacokinetics may also serve as a surrogate measurement of nasal deposition and absorption when a concomitant lack of pulmonary delivery is observed [106]. However, although indirect quantification methods enable comparisons between different nasal administration techniques, they do not enable IVIVC.

3.3. Biological effect measurement methods

Beyond nasal deposition, direct biological effects or measurements of surrogate markers of such effects represent the final clinical objective of *in vivo* evaluation. Such biological effects to be measured are as numerous as the clinical targets associated with nasal drug delivery, reaching from local to regional and systemic effects. First, nasal tissue is relatively simple to biopsy, which may enable direct histological evaluation of drug effects on epithelial remodeling and inflammation, for example [107] Local effects of drugs can also be evaluated macroscopically, through endoscopic examination (e.g., polyp grading [108]) or functionally, through standardized olfactory function testing [89]. Sinus drug deposition has also been evaluated, indirectly measuring exhaled nitric oxide after nebulizing a nitric oxide synthase inhibitor in the nose [87]. Patients symptom relief after drug administration can be evaluated through patient-centered symptom scoring systems, such as the sino-nasal outcome test (SNOT) [109], the medical outcomes study sleep scale (MOS-SS) or the morning congestion score [108]. When assessing effects of nose-to-brain drug delivery, direct biological measurements are not feasible in humans. Therefore, indirect assessments are required, such as quantification of cerebral blood flow changes using magnetic imaging

[57, 110] after insulin administration. In addition, the patients' cerebral symptoms scoring may also be performed, such as in nasal migraine therapy evaluation [111].

4. In vitro – In vivo correlation

In the field of intranasal aerosols, IVIVC can be analyzed separately in terms of the global aerosol deposited in the nose, as well as in terms of the regional deposition within the nasal cavities.

Total nasal deposition is generally assessed to compare the efficacy of different nasal aerosol delivery devices to predominantly target the nasal cavity comparatively to the lower respiratory tract. The nasal deposition is, therefore, described as a filtration efficiency function [112, 113]. Nasal pumps are designed for strict nasal drug deposition, with droplets sizes significantly larger than 10 µm, as required by regulatory bodies. Many studies have already investigated the relationship between the *in vitro* measured aerosol DSD and *in vivo* nasal aerosol deposition. Indeed, it is well established that *in vivo* nasal deposition increases with particle size and velocity [64, 112, 114], which has also been observed in *in vitro* studies [46, 115]. For example, Moller et al [30] have observed 100% of total nasal deposition with large droplets (60 µm) and only 70% with smaller droplets (3.5 µm). Most of these studies show a good IVIVC; however, some *in vivo* results are in contradiction with expected *in vitro* prediction based solely on particle size measurements [115]. Recently, Vecellio *et al.* [99] observed that a droplet size inferior to 10 µm can be associated with total nasal retention of a nasally inhaled aerosol without any aerosol penetrating the lung. Moreover, Djuspesland observed similar poor IVIVC among 16 healthy volunteers, with two 3.5 µm droplet-sized aerosols produced by two types of nasal devices [100]. One aerosol was generated in one nostril by blowing into the device during an oral exhalation, while the other aerosol was generated during a nasal inhalation. These two conditions generate different deposition profiles, illustrating the importance of airflow impact on nasal deposition.

Given these *in vivo* studies, the DSD should not be considered alone to make an *in vivo* deposition prediction. Although outside the scope of regulatory requirements, it appears interesting to consider the *in vitro* airflow supply when evaluating nasally inhaled aerosols and its influence on nasal deposition. In particular, in cases where the anatomic geometry is strongly restricted, such as in children's nasal cavities, airflow and geometry may act in synergy to greatly amplify impaction phenomena in the nose of even micrometric particles.

Concerning regional nasal deposition, most *in vitro* studies were performed on anatomical models. However, this tool does not measure any aerosol characteristic per se, as it describes and quantifies an aerosol deposition pattern.

A great number of *in vitro* studies using different types of anatomical models [78, 70, 89, 99, 60] as well as *in vivo* studies, have observed good IVIVC by measuring the deposition of radiolabeled aerosols [98, 99, 116], or by indirectly measuring biological effects such nasal resistance [117]. However, large and fast droplets as measured by *in vitro* studies can also have a deep penetration into the nasal cavity if one pays attention to specific implementation details, such as the angle and the insertion depth of the nasal device or the formulation viscosity [40]. However, it has been demonstrated that the plume geometry pattern of two different nasal spray formulations shows a poor IVIVC in terms of the expected deposition in the turbinates of healthy volunteers [106]. Spray formulations in development are usually tested for deposition in coated nasal models [57, 37] without *in vivo* deposition validation. The formulation of an aerosol (powder, liquid, gel) may also influence its physical characteristics and, therefore, its deposition. A gel form of aerosol sprayed in a nasal anatomical model, coated with medical lubricant, exhibited a higher deposition in the upper turbinate, in comparison with a non-gel control solution with normal viscosity [42]. Interestingly, a similar turbinate deposition was observed *in vivo* with a comparable gel formulation in healthy volunteers, quantified by endoscopy detection of fluorescein in comparison with a saline spray [94].

Many studies have focused on targeting maxillary sinuses (MS) aerosol deposition. However, no previously described *in vitro* characteristics would predict the penetration and deposition of aerosol droplets into the maxillary sinuses without testing the deposition in an anatomical model. Sinus aerosol deposition can be achieved by modifying air flow [29, 87]. Most *in vitro* studies were carried out in anatomical models of various geometries [31, 81, 67, 88, 93, 68]. Moreover, a reasonably good correlation has been observed with *in vivo* deposition study evaluating laminar flow [69, 30, 99]. Furthermore, a positive correlation may be assumed, given the favorable clinical studies observing positive results on clinical outcomes, such as for SNOT, nasal secretion analysis or nitric oxide measurements [87, 109, 118, 119]. In addition, *in vivo* and *in vitro* data are in agreement, showing that large particles, such as spray droplets, do not reach the maxillary sinuses [30, 67, 120]. In terms of exact

quantification of aerosol deposited in anatomical regions, IVIVC was observed for one anatomical model [69].

Global and regional aerosol deposition in the nose is also influenced by the specific conditions of use by the patient, despite consistency in spray characteristics and administration parameters (depth, angle). Indeed, Homer *et al.* observed that vigorously inhaling whilst administering a spray made no difference in terms of its distribution in the turbinate region, in comparison with no inhalation [121]. When regarding the patient position (supine vs. upright position), significant differences were observed in terms of deposition pattern of 50 µm sprays [102].

Despite some favorable results, IVIVC could be strongly questioned when considering anatomical characteristic features as influent parameters. First, with regards to the level of *in vivo* representativeness of anatomical nasal models and the modelling method, the results of IVIVC may not be the same, depending on the selected model used for *in vitro* deposition analysis [69, 38]. Moreover, some limits in *in vivo* prediction should be considered with *in vitro* data deposition obtained from models with sinus volumes which were too small [29] or with very smooth and reduced idealized ethmoid geometry [48]. Creating a standard geometry tool has been an interesting approach to standardize *in vitro* nasal deposition analysis, but it could strongly decrease the *in vivo* inter-subject variation, also impacting IVIVC. Recent studies have taken into account these anatomical specifications by increasing modelling accuracy, for either ethmoid or for maxillary sinuses [50, 51, 53, 73].

Finally, the internal nasal geometry can be summarized and considered as a tangle of different volumes and surfaces. In the field of nasal treatments, patients can present conditions such as ostium blockage, purulent secretions, nasal polyps, synechiae, turgescent turbinate and septal deviation, etc. In such cases, the surface area to volume ratio is strongly disturbed in comparison with healthy anatomy. Therefore, the *in vitro* aerosol characteristics that influence *in vivo* deposition in healthy subjects would not have the same behavior in patients. A small velocity of particles would increase along restricted passages. For instance, deep deposition of aerosols *in vitro* could be reduced *in vivo*, due to the presence of nasal polyps. Indeed, *in vitro* modelling of nasal cavities is limited by the lack of pathological models and consequently a lack of *in vitro* patients. In this case, a good IVIVC obtained

between an "*in vitro* healthy subject" and an "*in vivo* healthy volunteer" should not support a good prediction for the "*in vivo* patient."

The modelling of nasal cavities by 3D-printing led to the creation of a new tool to measure intranasal deposition *in vitro* according to a large panel of characteristics. The measure of nasal aerosol deposition in these models is now quasi-systematically included in the panel of *in vitro* characterizations, on top of standard regulatory methods, as a preclinical requirement for *in vivo* studies. The aim of this complete pre-clinical evaluation is to personalize and design the optimal nasal treatment (formulation, administration, etc.) efficient for each individual patient [56], or to select the optimized setting to be used in the *in vivo* study [57].

Most of the time, users directly consider the nasal model deposition as a quasi *in vivo* result, on the grounds that the 3D anatomical model is built from human CT-scan. Thus, only a few studies have elaborated an experimental set up to formally test IVIVC implementing the exact same delivery and measurement setup *in vitro* and *in vivo* [69, 87].

In short, an improvement in accuracy of the *in vivo* prediction will be gained by overlapping the different sources of *in vitro* data.

5. Expert opinion

5.1 Parameters influencing nasal deposition

Three key parameters have been identified to influence total nasal deposition efficiency: particle characteristics, ventilation and nasal anatomy.

Moreover, 10 µm is the limit of particle size reported by regulatory bodies, FDA and EMEA [7, 8] and nasal studies [38] to avoid lung deposition of nasal products. Deposition efficiency in the whole nasal cavities for aerosol particles larger than 1µm is governed by inertial impaction described by the Stokes number, which is a function of the aerodynamic diameter, air flow rate, and the characteristic nasal airway dimension [114]. Particles larger than 10µm generated by nasal sprays are also governed by the Stokes number, but high Stokes numbers (particles larger 20µm) could impact the sensitivity to other physical parameters, such as injection velocity, insertion angle, and spray cone angle on deposition distribution [23]. Indeed, the 10µm particle size limit has been commonly accepted; however, it remains questionable regarding new nasal devices using fine particles without lung deposition as described above [99, 100]. Moreover, this particle size limit has also raised the question regarding the difference between an aerosol and a nasal spray. Specifically, particles in a nasal spray have a higher particle velocity than aerosols and, consequently, a higher deposition efficacy for a similar particle size. Therefore, a unique limit of 10µm is questionable for a nasal spray.

The influence of air flow on nasal deposition has been clearly demonstrated by *in vivo* and anatomical model studies with nebulizers. However, the value of air flow is not the only parameter that needs to be considered: the direction of the air flow can also impact nasal and lung deposition.

The effects of nasal anatomy on nasal deposition have been demonstrated with an *in vitro* anatomical model and *in vivo* deposition studies [103]. One computational fluid dynamics study performed on 16 different anatomical nostrils demonstrated that olfactory deposition ranged from 4% to 100% when the injection location and spray characteristics were chosen to maximize olfactory deposition (particle diameter: 5-40 µm; particle velocity: 0-20m/sec; spray half cone angle: 17.5 to 30°, spray cone direction: upward to semi upward) [122].

Moreover, an *in vitro* study using 10 realistic nasal models showed a decrease of 73% to 11% of cromolyn sodium deposition in the turbinate region with the increase of the administration angle from 30° to 75°. Moreover, correlation was also observed between the turbinate deposition, the minimum cross-sectional area of the models and the length of the models [56].

More recently, deposition in 5 realistic nasal casts for two different device orientations were examined by researchers at the University of Alberta in Canada [123]. Authors measured deposition in anterior turbinates from 25% to 65% and from 15% to 50% for 45° and 60° spray angles respectively. Moreover, the study's authors concluded that the idealized replica agreed well with average regional deposition in the realistic replicas. Deposition variability was higher with realistic replicas than with the idealized replica. Additional studies may be required with different nasal sprays and aerosols to validate the idealized model, which does not include sinuses [114].

A study examining *in vivo* deposition using a nasal spray on 10 volunteers has reported 91% variability in terms of deposition distribution between the upper and lower parts of the nasal cavities, and 92% variability between their inner and outer parts [116]. Furthermore, a large variability (around 60%) for maxillary sinuses deposition was also observed when using a jet nebulizer on 6 healthy volunteers [99].

5.2 Unmet need for nasal drug IVIVC

IVIVC are generally based on few cases or represent mainly qualitative comparisons. The lack of strong IVIVC for nasal deposition can be explained by several different factors.

First, there is a lack of *in vivo* studies evaluating the influence of physical parameters on deposition patterns in nasal cavities, thus also limiting evaluation of IVIVC. During inhalation drug development, lung deposition is often measured before the clinical trial, which is generally not the case with nasal drugs, except for highly innovative devices. Based on this consideration, we recommend for the deposition measurements of new drugs to be performed before clinical trials to further elucidate their clinical drug efficacy.

However, *in vivo* deposition studies require validated measurement methods in order to validate high quality results. For example, there is a need to standardize scintigraphy methods for nasal deposition measurement. Relationships between anatomical regions and pharmacokinetic or clinical effect are unclear to date. Scientific studies including deposition measurements and clinical effect evaluation should be conducted similarly to the inhalation studies performed for bronchodilators [124].

The lack of strong IVIVC can also be explained by the limitation of *in vitro* measurement methods. Standardized methods are not always adapted for new nasal drug delivery systems, which questions the relevance of these methods. Nasal sprays generate inhomogeneous particle sizes, as well as concentrations and velocities in the air; furthermore, their characteristics change over the time of the spray generation. Furthermore, the use of active compounds in the form of a suspension adds complexity to this situation, as the drug concentrations inside droplets could be different. Regarding anatomical models to mimic *in vivo* deposition, technological advances could help to further improve IVIVC. But once again, key parameters will need to be defined to limit the complexity of the models.

5.3 Scientific key driver to improve IVIVC

5.3.1 The use of physics for identification of key parameters to characterize nasal drug delivery

Physics equations can be used to model the kinetics of particles, and thus, the deposition distribution. Based on this consideration, the use of physics formulas should help to select the most important parameters to be measured by *in vitro* methods. For example, for inhaled aerosol, the Stokes number is the key parameter to predict deposition by impaction [114]. It takes into account the DSD, the air velocity and the nasal airway dimension. Since sprays and aerosols are different in terms of generation and kinetics, this should be evaluated through the examination of physical impact of those differences and taken into account when performing measurements (when the spray is not fully developed for example).

5.3.2. The use of standardized ROIs

Nasal cavities are complex structures with different geometries. Therefore, ROIs used in studies should be precisely defined with regards to the therapeutic area. Moreover, further studies are necessary to evaluate the influence precise location of the drug deposition on the clinical efficacy. These measures will help to standardize ROIs in nasal cavities, as well as in central lungs for bronchodilator inhalation.

5.3.3. Consideration of the large *in vivo* variability vs. the *in vitro* standardized methods

When an IVIVC evaluation is conducted, the *in vivo* variability is generally higher than the *in vitro* variability (around 100% in terms of in vivo deposition variability in specific anatomical region vs. 10% for spray characteristic measurement [116]). Therefore, *in vitro* measurements should be performed, while taking into consideration this *in vivo* variability as best as possible. Regarding particle characteristic measurement, it seems difficult to take the *in vivo* variability into consideration. Nasal cast models seem more suitable to reproduce these variabilities and could thus be more relevant for IVIVC evaluation.

5.3.4. Formulation consideration for nasal deposition

Particles can be solid or liquid, with different chemical and physical properties. Accordingly, standard deposition models consider the particle in terms of its aerodynamic diameter or geometric diameter to predict its deposition with no impact of formulation on its deposition.

Indeed, this is theoretically true; however, formulation should be considered for IVIVC evaluation regarding the large delivered doses and the rapid change in drug compounds after deposition. The importance of this point can be explained by the total mass of deposited particles per area. If we compare nasal deposition and lung deposition in terms of deposited mass per area, we obtain the value of approximately 1mg/cm^2 for nasal deposition, whereas we obtain the value of less than 0.1µg/cm^2 for lung deposition. Consequently, there is a 1000- fold higher mass of drug per cm^2 in nasal cavities compared to the lung, explaining, for example why dripping is observed after nasal drug deposition. Based on this consideration, the deposited mass of particles can act like an anatomical geometry modification, reducing the passage for other particles, and thus, increasing the deposition in this deposited region. The bounce of the deposited powder should also be

considered with nasal powder devices for the same reason. Moreover, liquid formulations can also quickly modify the surface recovery after deposition. Using a liquid with a high surface tension could increase the surface of recovery after deposition, and thus, modify rapidly the initial deposition of droplets.

6. Conclusion

Particles larger than 10 µm are deposited in nasal cavities, but particles smaller than this size may not necessarily penetrate the lungs. Deposition in nasal cavities depends on physical particle characteristics, ventilation and, patient anatomy. The lack of strong IVIVC for deposition patterns in the different regions of nasal cavities could be improved with the emergence of improved anatomical models. Therefore, it is necessary to develop relevant *in vitro* and *in vivo* measurement methods and to improve our knowledge regarding the nasal deposition mechanisms.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

List of figure legends

References

[1] B.L. Laube, Devices for aerosol delivery to treat sinusitis, J Aerosol Med. 20 Suppl. 1(2007) S5-17.

[2] A. Pires, A. Fortuna, G. Alves, A. Falcão, Intranasal drug delivery: how, why and what for? J Pharm Pharm Sci. 12 (2009) 288-311.

[3] S. Gizurarson, Anatomical and histological factors affecting intranasal drug and vaccine delivery, Curr Drug Deliv. 9 (2012) 566-82.

[4] P.G. Djupesland, J.C. Messina, R.A. Mahmoud, The nasal approach to delivering treatment for brain diseases: an anatomic, physiologic, and delivery technology overview, Ther Deliv. 5 (2014) 709-33.

[5] J. Dugernier, G. Reychler, L. Vecellio, S. Ehrmann, Nasal High-Flow Nebulization for Lung Drug Delivery: Theoretical, Experimental, and Clinical Application. J Aerosol Med Pulm Drug Deliv. 32 (2019) 341-351.

[6] K. Wiesmiller, T. Keck, R. Leiacker, T. Sikora, G. Rettinger, J. Lindemann, The impact of expiration on particle deposition within the nasal cavity, Clin Otolaryngol Allied Sci. 28 (2003) 304-307.

[7] Guideline on the pharmaceutical quality of inhalation and nasal products, 2006, EMEA

[8] GUIDANCE DOCUMENT. Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products--Chemistry, Manufacturing, and Controls Documentation Guidance for Industry July 2002, FDA

[9] Nasal Preparations/Nasalia, European Pharmacopoeia, 3rd ed., 1997, Council of Europe, Strasbourg, pp. 1763-1765.

[10] Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action April 2003, Guidance document, FDA.

[11] W.H. Doub, W.P. Adams, A.M. Wokovich, J.C. Black, M. Shen, L.F. Buhse, Measurement of drug in small particles from aqueous nasal sprays by Andersen Cascade Impactor, Pharm Res. 29 (2012) 3122-3130.

[12] G. Williams, C. Blatchford, J.P. Mitchell, Evaluation of Nasal Inlet Ports Having Simplified Geometry for the Pharmacopeial Assessment of Mass Fraction of Dose Likely to Penetrate Beyond the Nasopharynx: A Preliminary Investigation, AAPS PharmSciTech. 19 (2018) 3723-3733.

[13] J. Grmaš, R. Dreu, R. Injac, Analytical Challenges of Spray Pattern Method Development for Purposes of *In vitro* Bioequivalence Testing in the Case of a Nasal Spray Product, J Aerosol Med Pulm Drug Deliv. 32 (2019) 200-212.

[14] S. Trows, K. Wuchner, R. Spycher, H. Steckel, Analytical challenges and regulatory requirements for nasal drug products in Europe and the U.S. Pharmaceutics. 6 (2014) 195-219.

[15] C. Guo, W.H. Doub, The influence of actuation parameters on *in vitro* testing of nasal spray products, J Pharm Sci. 9 (2006) 2029-2040.

[16] P. Kippax, J. Suman, Applications for Droplet Sizing. Manual versus Automated Actuation of Nasal Sprays, Pharmaceutical Technology. (2004) 30-39.

[17] D.V. Doughty, W. Hsu, R.N. Dalby, Automated actuation of nasal spray products: effect of hand-related variability on the *in vitro* performance of Flonase nasal spray, Drug Dev Ind Pharm. 40 (2014) 711-718.

[18] P. Farjas, A. Regard, G. Grevin, Influence of the Human Factor on Nasal Drug Delivery Device Evaluation, ONdrugDelivery Magazine. 85 (2018) 4-8.

[19] Q Liu, M Absar, B Saluja, C Guo, B Chowdhury, R Lionberger, DP Conner, BV Li. J. Scientific Considerations for the Review and Approval of First Generic Mometasone Furoate Nasal Suspension Spray in the United States from the Bioequivalence Perspective. AAPS 21 (2019) 14.

[20] EN ISO 27427. Anaesthetic and respiratory equipment - Nebulizing systems and components (2013).

[21] D. Moraga-Espinoza, Z. Warnken, A. Moore, R.O. Williams, H.D.C. Smyth, A modified USP induction port to characterize nasal spray plume geometry and predict turbinate deposition under flow. Int J Pharm. 548 (2018) 305-313.

[22] X. Liu, W.H. Doub, C. Guo, Assessment of the influence factors on nasal spray droplet velocity using phase-Doppler anemometry (PDA), AAPS PharmSciTech. 12 (2011) 337-343.

[23] K. Inthavong, M.C. Fung, W. Yang, J. Tu, Measurements of droplet size distribution and analysis of nasal spray atomization from different actuation pressure, J Aerosol Med Pulm Drug Deliv. 28 (2015) 59-67.

[24] R. Badre, R. Guillerm, Les aerosols, In: L. Flottes, P. Clerc, R. Riu, F. Devilla, (Librairie Arnette) La physiologie des sinues et ses applications cliniques et thérapeutiques, Societe française d'otorhinolaryngologie, (1959) 522-535.

[25] R. Guillerm, R. Badre, L. Flottes, R. Riu, A. Rey, A new method of aerosol penetration into the sinuses., Presse Med. 67 (1959) 1097–1098.

[26] C.S. Kim, M.A. Eldridge, Aerosol deposition in the airway with excessive mucus, J. Appl. Physiol. 59 (1985)1766-1772.

[27] M. Maniscalco, E. Weitzberg, J. Sundberg, M. Sofia, J.O. Lundberg. Assessment of nasal and sinus nitric oxide output using single-breath humming exhalations. Eur. Respir. J. 22 (2003) 323-329.

[28] M. Durand, L. Vecellio, G. Aubert, G. Chantrel, J. M. Prades, *In vitro* study of sonic aerosol to maxillary sinuses treatment, Drug Delivery to the Lungs (2005).

[29] W. Möller, U. Schuschnig, G. Meyer, H. Mentzel, M. Keller, Ventilation and drug delivery to the paranasal sinuses: Studies in a nasal cast using pulsating airflow, Rhinology. 46 (2008) 213–220.

[30] W. Möller, U. Schuschnig, G. Khadem Saba, G. Meyer, B. Junge-Hülsing, M. Keller, K. Häussinger, Pulsating aerosols for drug delivery to the sinuses in healthy volunteers, Otolaryngol. - Head Neck Surg. 142 (2010) 382–388.

[31] S.F. Thieme, W. Möller, S. Becker, U. Schuschnig, O. Eickelberg, A.D. Helck, M.F. Reiser, T.R.C. Johnson, Ventilation imaging of the paranasal sinuses using xenon-enhanced dynamic single-energy CT and dual-energy CT: A feasibility study in a nasal cast, Eur. Radiol. 22 (2012) 2110–2116.

[32] W. Möller, G. Celik, S. Feng, P. Bartenstein, G. Meyer, O. Eickelberg, O. Schmid, S. Tatkov, Nasal high flow clears anatomical dead space in upper airway models, J. Appl. Physiol. 118 (2015) 1525–1532.

[33] J. Xi, X.A Si, S. Peters, D. Nevorski, T. Wen, M. Lehman. Understanding the mechanisms underlying pulsating aerosol delivery to the maxillary sinus: In vitro tests and computational simulations, Int J Pharm. 30 (2017) 520: 254-266.

[34] D.L. Swift, Inspiratory inertial deposition of aerosols in human nasal airway replicate casts: Implication for the proposed NCRP lung model, Radiat. Prot. Dosimetry. 38 (1991) 29-34.

[35] R.A. Guilmette, and T.J. Gagliano. Construction of a model of human nasal airways using in vivo morphometric data, Ann. Occup. Hyg, 38 (1994) 69–75.

[36] Y.S. Cheng, T.D. Holmes, J. Gao, R.A. Guilmette, S. Li, Y. Surakitbanharn, and C. Rowlings. characterization of nasal spray pumps and deposition pattern in a replica of the human nasal airway. J. Aerosol. Med. 14 (2001) 267–280.

[37] M.Y. Foo, Y.S. Cheng, W.C. Su, M.D. Donovan, The influence of spray properties on intranasal deposition, J. Aerosol Med. Depos. Clear. Eff. Lung. 20 (2007) 495–508.

[38] J.T. Kelly, B. Asgharian, J.S. Kimbell, B.A. Wong, Particle deposition in human nasal airway replicas manufactured by different methods. Part I: Inertial regime particles, Aerosol Sci. Technol. 38 (2004) 1063–1071.

[39] V. Kundoor, R.N. Dalby, Assessment of nasal spray deposition pattern in a silicone human nose model using a color-based method, Pharm. Res. 27 (2010) 30–36.

[40] V. Kundoor, R.N. Dalby, Effect of formulation- and administration-related variables on deposition pattern of nasal spray pumps evaluated using a nasal cast, Pharm. Res. 28 (2011) 1895–1904.

[41] Y. Guo, B. Laube, R. Dalby, The effect of formulation variables and breathing patterns on the site of nasal deposition in an anatomically correct model, Pharm. Res. 22 (2005) 1871–1878.

[42] J. Castile, Y.-H. Cheng, B. Simmons, M. Perelman, A. Smith, P. Watts, Development of *in vitro* models to demonstrate the ability of PecSys®, an in situ nasal gelling technology, to reduce nasal run-off and drip., Drug Dev. Ind. Pharm. 39 (2013) 816–24.

[43] S. Lungare, J. Bowen, R. Badhan, Development and Evaluation of a Novel Intranasal Spray for the Delivery of Amantadine., J. Pharm. Sci. 105 (2016) 1209–20.

[44] L. Nižić , I. Ugrina, D. Špoljarić, V. Saršon, M.S. Kučuk, I. Pepić, A. Hafner. Innovative sprayable in situ gelling fluticasone suspension: Development and optimization of nasal deposition. Int J Pharm. 536 (2019) 445-456.

[45] R. Hughes, J. Watterson, C. Dickens, D. Ward, A. Banaszek, Development of a nasal cast model to test medicinal nasal devices., Proc. Inst. Mech. Eng. H. 222 (2008) 1013–22.

[46] S.A. Shah, C.J. Dickens, D.J. Ward, A.A. Banaszek, C. George, W. Horodnik, Design of experiments to optimize an *in vitro* cast to predict human nasal drug deposition, J. Aerosol Med. Pulm. Drug Deliv. 27 (2014) 21–29.

[47] R.S. Kaye, T.S. Purewal, O.H. Alpar, Development and testing of particulate formulations for the nasal delivery of antibodies, J. Control. Release. 135 (2009) 127–135.

[48] E. Javaheri, L. Golshahi, W. H. Finlay, An idealized geometry that mimics average infant nasal airway deposition, Journal of Aerosol Science, 55 (2013) 137-148E.

[49] J.D. Schroeter, E.W. Tewksbury, B.A. Wong, J.S. Kimbell, Experimental measurements and computational predictions of regional particle deposition in a sectional nasal model, J. Aerosol Med. Pulm. Drug Deliv. 28 (2015) 20–29.

[50] J. Xi, J.E. Yuan, Y. Zhang, D. Nevorski, Z. Wang, Y. Zhou, Visualization and Quantification of Nasal and Olfactory Deposition in a Sectional Adult Nasal Airway Cast., Pharm. Res. 33 (2016) 1527-1541.

[51] S.B. Yarragudi, R. Richter, H. Lee, G.F. Walker, A.N. Clarkson, H. Kumar, S.B. Rizwan, Formulation of olfactory-targeted microparticles with tamarind seed polysaccharide to improve nose-to-brain transport of drugs., Carbohydr. Polym. 163 (2017) 216–226.

[52] T. Okuda, P. Tang, J. Yu, W.H. Finlay, H.K. Chan, Powder aerosol delivery through nasal high-flow system: *In vitro* feasibility and influence of process conditions, Int. J. Pharm. 533 (2017) 187–197.

[53] J. Xi, Z. Wang, D. Nevorski, T. White, Y. Zhou, Nasal and Olfactory Deposition with Normal and Bidirectional Intranasal Delivery Techniques: *In vitro* Tests and Numerical Simulations, J. Aerosol Med. Pulm. Drug Deliv. 30 (2017) 118–131

[54] N. Sawant, M.D. Donovan, *In vitro* Assessment of Spray Deposition Patterns in a Pediatric (12 Year-Old) Nasal Cavity Model, Pharm. Res. (2018).

[55] M. Azimi, G. Williams, L. Golshahi, P. W. Longest, M. Hindle, Evaluating simulated patient use in realistic nasal airway models for the *in vitro* characterization of nasal spray products, Inhalation (2018) 11-17.

[56] Z.N. Warnken, H.D.C. Smyth, D.A. Davis, S. Weitman, J.G. Kuhn, R.O. Williams, Personalized Medicine in Nasal Delivery: The Use of Patient-Specific Administration Parameters to Improve Nasal Drug Targeting Using 3D-Printed Nasal Replica Casts, Mol. Pharm. 15 (2018) 1392-1402.

[57] J. Wingrove, M. Swedrowska, R. Scherließ, M. Parry, M. Ramjeeawon, D. Taylor, G. Gauthier, L. Brown, S. Amiel, F. Zelaya, B. Forbes, Characterisation of nasal devices for delivery of insulin to the brain and evaluation in humans using functional magnetic resonance imaging., J. Control. Release. 302 (2019) 140-147.

[58] C.J.T. Spence, N.A. Buchmann, M.C. Jermy, S.M. Moore, Stereoscopic PIV measurements of flow in the nasal cavity with high flow therapy, in: Exp. Fluids, 2011: pp. 1005–1017.

[59] RDD website https://www.rddonline.com/rdd/rdd.php?sid=105, *last accessed on 2020, July 30th.*

[60] R. Saijo, Y. Majima, N. Hyo, H. Takano, Particle deposition of therapeutic aerosols in the nose and paranasal sinuses after transnasal sinus surgery: A cast model study, Am. J. Rhinol. 18 (2004) 1–7.

[61] H.M. Janssens, J.C. De Jongste, W.J. Fokkens, S.G.F. Robben, K. Wouters, H.A.W.M. Tiddens, The Sophia anatomical infant nose-throat (saint) model: A valuable tool to study aerosol deposition in infants, J. Aerosol Med. Depos. Clear. Eff. Lung. 14 (2001) 433–441.

[62] B.L. Laube, G. Sharpless, C. Shermer, O. Nasir, V. Sullivan, K. Powell, Deposition of albuterol aerosol generated by pneumatic nebulizer in the Sophia Anatomical Infant Nose-Throat (SAINT) model, Pharm. Res. 27 (2010) 1722–1729.

[63] F. Réminiac, L. Vecellio, R. Mac Loughlin, D. Le Pennec, M. Cabrera, N.H. Vourc'h, J.B. Fink, S. Ehrmann, Nasal high flow nebulization in infants and toddlers: An *in vitro* and *in vivo* scintigraphic study, Pediatr. Pulmonol. 52 (2017) 337–344.

[64] J. Storey-Bishoff, M.Noga, W. H. Finlay, Deposition of micrometer-sized aerosol particles in infant nasal airway replicas, Journal of Aerosol Science, 39 (2008) 1055-1065.

[65] S. Minocchieri, J.M. Burren, M.A Bachmann, G. Stern, J. Wildhaber, S. Buob, R. Schindel, R. Kraemer, U.P. Frey, M. Nelle. Development of the premature infant nose throat-model (PrINT-Model): an upper airway replica of a premature neonate for the study of aerosol delivery. Pediatr Res. 64 (2008) 141-6.

[66] S. Le Guellec, S. Gatier, D. Le Pennec, F. Vullo, G. Chantrel, L. Vecellio, Scintigraphic evaluation of the Easynose nasal nebuliser in a nasal cast model. Drug Delivery to the Lungs (2011).

[67] L. Leclerc, J. Pourchez, G. Aubert, S. Leguellec, L. Vecellio, M. Cottier, M. Durand, Impact of airborne particle size, acoustic airflow and breathing pattern on delivery of nebulized antibiotic into the maxillary sinuses using a realistic human nasal replica, Pharm. Res. 31 (2014) 2335–2343.

[68] A. El Merhie, L. Navarro, X. Delavenne, L. Leclerc, J. Pourchez, A new strategy to improve drug delivery to the maxillary sinuses: The frequency sweep acoustic airflow, Pharm. Res. 33 (2016) 1074–1084.

[69] S. Le Guellec, D. Le Pennec, S. Gatier, L. Leclerc, M. Cabrera, J. Pourchez, P. Diot, G. Reychler, L. Pitance, M. Durand, F. Jamar, L. Vecellio, Validation of anatomical models to study aerosol deposition in human nasal cavities, Pharm. Res. 31 (2014) 228–237.

[70] M. Francis, D. Le Pennec, G. Williams, E. Duclos, P. Diot, L. Vecellio, *in vitro* study of aerosol deposition in nasal cavities during inhalation and exhalation, Respiratory Drug Delivery (2013).

[71] L. Salade, N. Wauthoz, J. Goole, K. Amighi, How to characterize a nasal product. The state of the art of *in vitro* and ex vivo specific methods, Int J Pharm. 561 (2019) 47-65.

[72] T. Sartoretti, M. Mannil, S. Biendl, J.M. Froehlich, H. Alkadhi, M. Zadory, *In vitro* qualitative and quantitative CT assessment of iodinated aerosol nasal deposition using a 3D-printed nasal replica, Eur. Radiol. Exp. 3 (2019) 32.

[73] S. Hosseini, X. Wei, J. V. Wilkins, C.P. Fergusson, R. Mohammadi, G. Vorona, L. Golshahi, *In vitro* Measurement of Regional Nasal Drug Delivery with Flonase,® Flonase® Sensimist,TM and MAD Nasal™ in Anatomically Correct Nasal Airway Replicas of Pediatric and Adult Human Subjects, J. Aerosol Med. Pulm. Drug Deliv, 32 (2019) : 374-385.

[74] The University of North Carolina (UNC), school medicine website (Chapel Hill), Center for Environmental Medicine, Asthma and Lung Biology, Mucociliary Clearance and Aerosol Research Laboratory ; https://www.med.unc.edu/cemalb/mucociliary-clearance/bench-research-studies. *Last accessed on 2020, July 30th.*

[75] 2019 CFD Research Corporation, https://www.cfd-research.com /products/nasal-cavity-phantoms/ *last accessed on 2020, july 30th.*

[76] M. B. St Martin, C.J. Hitzman, T.S. Wiedmann, & F.L. Rimell Deposition of aerosolized particles in the maxillary sinuses before and after endoscopic sinus surgery. American journal of rhinology, 21 (2007) 196–197.

[77] C. Hilton, T. Wiedmann, M. St Martin, B. Humphrey, R. Schleiffarth, F. Rimell, Differential deposition of aerosols in the maxillary sinus of human cadavers by particle size, Am. J. Rhinol. 22 (2008) 395–398.

[78] A. Moffa, A. Costantino, V. Rinaldi, L. Sabatino, E.M.C. Trecca, P. Baptista, P. Campisi, M. Cassano, M. Casale, Nasal delivery devices: A comparative study on cadaver model, Biomed Res. Int. 2019 (2019).

[79] R.J. Valentine, T. Athanasiadis, M. Thwin, D. Singhal, E.K. Weitzel, P.J. Wormald, A prospective controlled trial of pulsed nasal nebulizer in maximally dissected cadavers, Am. J. Rhinol. 22 (2008) 390–394.

[80] R. P. Manes, L. Tong, P.S. Batra. Prospective evaluation of aerosol delivery by a powered nasal nebulizer in the cadaver model. International forum of allergy & rhinology, 1(5) (2011) 366–371.

[81] M. Durand, P. Rusch, D. Granjon, G. Chantrel, J.M. Prades, F. Dubois, D. Esteve, J.F. Pouget, C. Martin, Preliminary study of the deposition of aerosol in the maxillary sinuses using a plastinated model, J Aerosol Med. 14 (2001) 83-93.

[82] C. Croce, R. Fodil, M. Durand, G. Sbirlea-Apiou, G. Caillibotte, J.F. Papon, J.R. Blondeau, A. Coste, D. Isabey, B. Louis, *In vitro* Experiments and Numerical Simulations of Airflow in Realistic Nasal Airway Geometry, Ann. Biomed. Eng. 34 (2006) 997–1007.

[83] M. Durand, J. Pourchez, B. Louis, J.F. Pouget, D. Isabey, A. Coste, J.M. Prades, P. Rusch, M. Cottier, Plastinated nasal model: a new concept of anatomically realistic cast., Rhinology. 49 (2011) 30–6.

[84] M. Durand, J. Pourchez, G. Aubert, S. Le Guellec, L. Navarro, V. Forest, P. Rusch, M. Cottier, Impact of acoustic airflow nebulization on intrasinus drug deposition of a human plastinated nasal cast: New insights into the mechanisms involved, Int. J. Pharm. 421 (2011) 63–71.

[85] L. Leclerc, A. El Merhie, L. Navarro, N. Prévôt, M. Durand, J. Pourchez, Impact of acoustic airflow on intrasinus drug deposition: New insights into the vibrating mode and the optimal acoustic frequency to enhance the delivery of nebulized antibiotic, Int. J. Pharm. 494 (2015) 227–234.

[86] Y. Zhou, M. Guo, J. Xi, H. Irshad, Y.S. Cheng, Nasal deposition in infants and children, J. Aerosol Med. Pulm. Drug Deliv. 27 (2014) 110–116.

[87] M. Maniscalco, M. Sofia, E. Weitzberg, J.O. Lundberg, Sounding airflow enhances aerosol delivery into the paranasal sinuses, Eur. J. Clin. Invest. 36 (2006) 509–513.

[88] Y. Sato, N. Hyo, M. Sato, H. Takano, S. Okuda, Intra-nasal distribution of aerosols with or without vibration, Z Erkr Atmungsorgane, 157 (1981) 276–280.

[89] G. Reychler, C. Colbrant, C. Huart, S. Le Guellec, L. Vecellio, G. Liistro, P. Rombaux, Effect of three-drug delivery modalities on Olfactory function in chronic sinusitis, Laryngoscope. 125 (2015) 549–555.

[90] M.Y. Foo, N. Sawant, E. Overholtzer, M.D Donovan, A Simplified Geometric Model to Predict Nasal Spray Deposition in Children and Adults, AAPS Pharm. Sci. Tech, 19 (2018) 2767-2777.

[91] J. Castile, Y.-H. Cheng, B. Simmons, M. Perelman, A. Smith, P. Watts, Development of *in vitro* models to demonstrate the ability of PecSys®, an in situ nasal gelling technology, to reduce nasal run-off and drip., Drug Dev. Ind. Pharm. 39 (2013) 816–24.

[92] N.B. Carrigy, C. O'Reilly, J. Schmitt, M. Noga, W.H. Finlay, Effect of facial material softness and applied force on face mask dead volume, face mask seal, and inhaled corticosteroid delivery through an idealized infant replica, J. Aerosol Med. Pulm. Drug Deliv. 27 (2014) 290–298.

[93] L. Leclerc, J. Pourchez, N. Prevot, L. Vecellio, S. Le Guellec, M. Cottier, M. Durand, Assessing sinus aerosol deposition: Benefits of SPECT-CT imaging, Int. J. Pharm. 462 (2014) 135–141.

[94] B.S. Bleier, I. Debnath, R.J. Harvey, R.J. Schlosser, Temporospatial quantification of fluorescein-labeled sinonasal irrigation delivery, Int Forum Allergy Rhinol. 1 (2011) 361-365

[95] P. Merkus, F.A. Ebbens, B. Muller, W.J. Fokken,. Influence of anatomy and head position on intranasal drug deposition, Eur Arch Otorhinolaryngol. 263 (2006) 827-832

[96] J.G. Hardy, S.W. Lee, C.G. Wilson, Intranasal drug delivery by spray and drops, J Pharm Pharmacol. 37 (1985) 294-297.

[97] I.A. Emanuel, M.S. Blaiss, E.O. Meltzer, P. Evans, A. Connor, Nasal deposition of ciclesonide nasal aerosol and mometasone aqueous nasal spray in allergic rhinitis patients, Am J Rhinol Allergy. 28 (2014) 117-121.

[98] P.G. Djupesland, A. Skretting, Nasal deposition and clearance in man: comparison of a bidirectional powder device and a traditional liquid spray pump, J. Aerosol Med. Pulm. Drug. Deliv, 280 (2012) 280-9.

[99] L. Vecellio, R. De Gersem, S. Le Guellec, G. Reychler, L. Pitance, D. Le Pennec, P. Diot, G. Chantrel, P. Bonfils, F. Jamar, Deposition of aerosols delivered by nasal route with jet and mesh nebulizers, Int. J. Pharm. 407 (2011) 87–94.

[100] P.G. Djupesland, A. Skretting, M. Winderen, T, Holand, Bi-directional nasal delivery of aerosols can prevent lung deposition, J Aerosol Med. 3 (2004) 249-259.

[101] P.G. Djupesland, A. Skretting, M. Winderen, T, Holand, Breath actuated device improves delivery to target sites beyond the nasal valve, Laryngoscope. 3 (2006) 466-472.

[102] R. Bacon, S. Newman, L. Rankin, G. Pitcairn, R. Whiting, Pulmonary and nasal deposition of ketorolac tromethamine solution (SPRIX) following intranasal administration, Int J Pharm. 431 (2012) 39-44.

[103] W. Möller, U. Schuschnig, G. Celik, W. Münzing, P. Bartenstein, K. Häussinger, W.G. Kreyling, M. Knoch, M. Canis, S. Becker, Topical drug delivery in chronic rhinosinusitis patients before and after sinus surgery using pulsating aerosols, PLoS One. 8 (2013)

[104] T.E. Corcoran, Imaging in Aerosol Medicine, Respir Care. 60 (2015) 850-855.

[105] A. Skretting, P.G. Djupesland, A new method for scintigraphic quantification of deposition and clearance in anatomical regions of the human nose. Nucl Med Commun. 30 (2009) 629-638.

[106] J.D. Suman, B.L. Laube, R. Dalby, Validity of *in vitro* tests on aqueous spray pumps as surrogates for nasal deposition, absorption, and biologic response, J Aerosol Med. 4 (2006) 510-521.

[107] C. Mastruzzo, L.R. Greco, K. Nakano, A. Nakano, F. Palermo, M.P. Pistorio, E.T. Salinaro, M. Jordana, J. Dolovich, D.N. Crimi, C. Vancheri, Impact of intranasal budesonide on immune inflammatory responses and epithelial remodeling in chronic upper airway inflammation, J Allergy Clin Immunol. 112 (2003) 37-44.

[108] R. Sindwani, J.K. Han, D.F. Soteres, J.C. Messina, J.L. Carothers, R.A. Mahmoud, P.G. Djupesland, NAVIGATE I: Randomized, Placebo-Controlled, Double-Blind Trial of the Exhalation Delivery System With Fluticasone for Chronic Rhinosinusitis With Nasal Polyps, Am J Rhinol Allergy. 33 (2019) 69-82.

[109] J.G. Mainz, I. Schiller, C. Ritschel, H.J. Mentzel, J. Riethmüller, A. Koitschev, G. Schneider, J.F. Beck, B. Wiedemann, Sinonasal inhalation of dornase alfa in CF: A double-blind placebo-controlled cross-over pilot trial, Auris Nasus Larynx. 38 (2011) 220-227.

[110] S. Kullmann, R. Veit, A. Peter, R. Pohmann, K. Scheffler, H.U. Häring, A. Fritsche, H. Preissl, M. Heni, Dose-Dependent Effects of Intranasal Insulin on Resting-State Brain Activity, J Clin Endocrinol Metab. 103 (2018) 253-262.

[111] R.B. Lipton, S. Munjal, E. Brand-Schieber, A.M. Rapoport, DFN-02 (Sumatriptan 10 mg With a Permeation Enhancer) Nasal Spray vs Placebo in the Acute Treatment of Migraine: A Double-Blind, Placebo-Controlled Study, Headache. 58 (2018) 676-687.

[112] M.H. Becquemin, D.L. Swift, A. Bouchikhi, M. Roy, A. Teillac, Particle deposition and resistance in the noses of adults and children, Eur Respir J, 4 (1991) 694-702.

[113] L. Golshahi, S. Hosseini S. Intranasal Filtration of Inhaled Aerosol in Human Subjects as a Function of Nasal Pressure Drop, J. Aerosol Med. Pulm. Drug Deliv. 32 (2019) 13-23.

[114] Y. S. Cheng, Aerosol Deposition in the Extrathoracic Region, Aerosol Sci Technol, 37 (2003) 659-671.

[115] S.P. Newman, G.R Pitcairn, R.N. Dalby, Drug delivery to the nasal cavity: *in vitro* and *in vivo* assessment, Drug Carrier Syst, 21 (2004) 21-66.

[116] J.D. Suman, B.L. Laube, R. Dalby, Comparison of nasal deposition and clearance of aerosol generated by nebulizer and an aqueous spray pump, Pharm Res. 16 (1999) 1648-1652.

[117] L.C. Goh, B. Arvin, A.B. Zulkiflee, N. Prepageran, Lidocaine/Phenylephrine Nasal Spray versus Nebulization Prior to Nasoendoscopy: A Randomized Controlled Trial, Otolaryngol. Head Neck Surg., 159 (2018) 783-788.

[118] O. Goektas, L. Lau, H. Olze, Treatment of chronic rhinosinusitis with pressure-pulsed corticosteroid inhalation, Indian J Otolaryngol Head Neck Surg. 65 (2013) 402-405

[119] W. J. Videler, C.M. van Drunen, J. B. Reitsma, W.J Fokkens, Nebulized bacitracin/colimycin: a treatment option in recalcitrant chronic rhinosinusitis with Staphylococcus aureus? A double-blind, randomized, placebo-controlled, cross-over pilot study. Rhinology, 46 (2008) 92-98.

[120] D. Senocak, M. Senocak, S. Bozan, Sinonasal distribution of topically applied particles: computerized tomographic detection and the effects of topical decongestion, Otolaryngol Head Neck Surg. 133 (2005) 944-948.

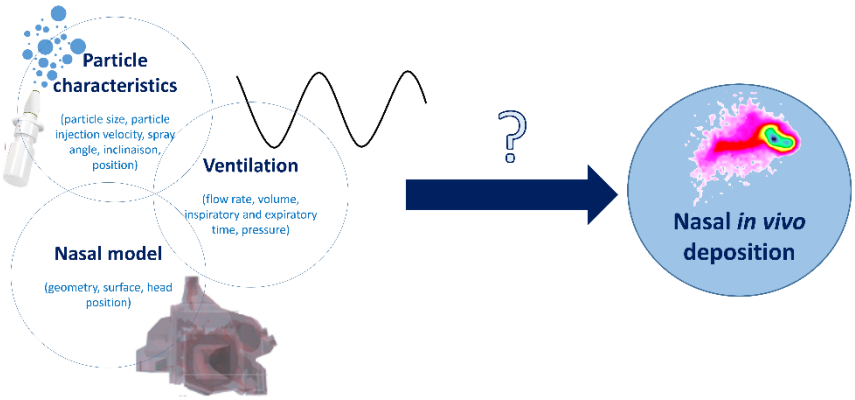
[121] J.J. Homer, C.H. Raine, An endoscopic photographic comparison of nasal drug delivery by aqueous spray, Clin Otolaryngol Allied Sci, 33 (1998) 560-3.

[122] M Kiaee, H Wachtel, ML Noga, AR Martin, WH Finlay, Regional deposition of nasal sprays in adults: A wide ranging computational study. Int J Numer Method Biomed Eng, 34 (2018) e2968.

[123] J.Z. Chen, M. Kiaee, A.R. Martin, W.H. Finlay. In vitro assessment of an idealized nose for nasal spray testing: Comparison with regional deposition in realistic nasal replicas. Int J Pharm, 582 (2020) 119341.

[124] O.S. Usmani, M.F. Biddiscombe, P.J. Barnes, Regional lung deposition and bronchodilator response as a function of beta2-agonist particle size, Am J Respir Crit Care Med. 172 (2005) 1497-1504.

List of figure legends



Abstract figure: List of physical parameters studied in previous *in vitro* studies that have an influence on *in vitro* nasal deposition.

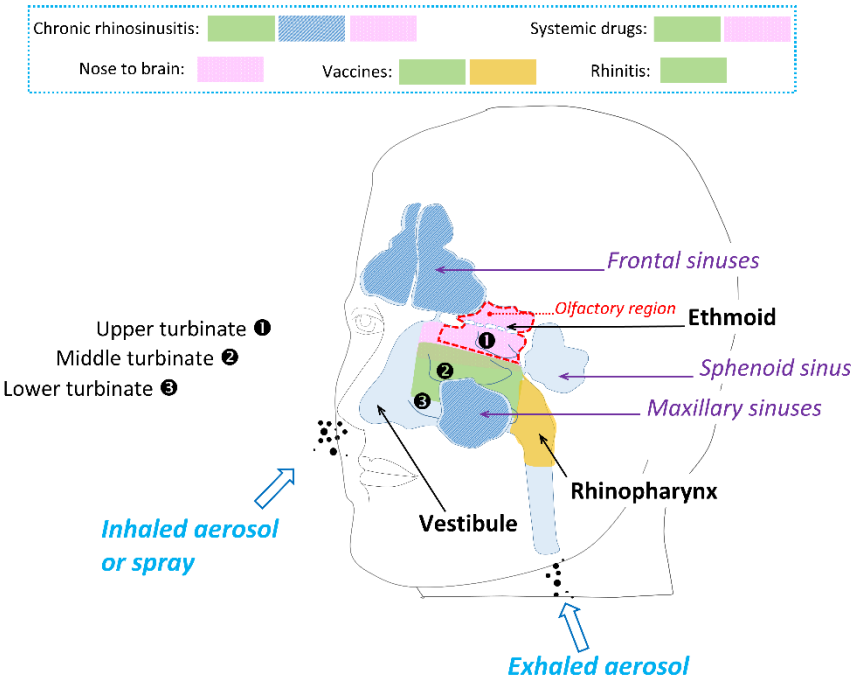


Figure 1: Schematic representation of adult nasal cavities and theoretical therapeutic target regions for nasal drug deposition.

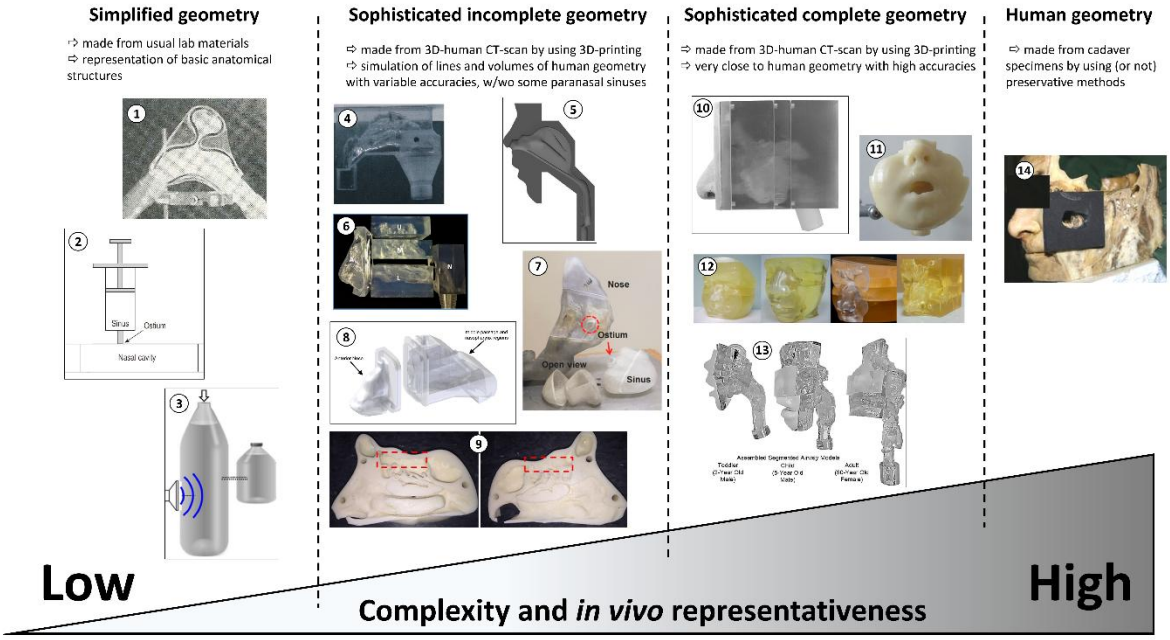


Figure 2: Classification proposition of *in vitro* anatomical models of nasal cavities in regards to the complexity and *in vivo* representativeness of *in vitro* geometry. The models images are shown as an example to illustrate the proposed classification. The circle number refers to the first column the Table 1. 3D: three dimensions, CT: computed tomography, w/wo: with or without. Illustration n°1 was adapted from [24] and republished with the French society of Otorhinolaryngology permission. Illustration n°2 was adapted from [27] and republished with the European Respiratory society permission. Illustration n° 3 was adapted from [33] and republished with permission by Copyright Clearance Center (CCC). Illustration n° 4 was republished with permission (CCC) from [38]. Illustration n° 5 was republished with permission (CCC) from [48]. Illustration n°6 was republished with permission (CCC) from [56]. Illustration n°7 was adapted from [33] and republished with permission (CCC). Illustration n°8 was republished with Inhalation magazine permission from [55]. Illustration n°9 was republished with permission (CCC) from [51]. Illustration n°10 is a laboratory photograph directly provided by authors, and published with APTARpharma, DTFmedical and University of Tours permissions. Illustration n°11 is a laboratory photograph directly provided by authors. Illustration n°12 was registered with permission from the CFD-Research Corporation website https://www.cfd-research.com/products/nasal-cavity-phantoms/, last accessed on 2020, July 30th. Illustration n°13 was adapted from [73] and republished with permission (CCC). Illustration n°14 is a laboratory photograph directly provided by the authors and published with permission by model holder.

Major points for IVIVC of intranasal deposition

- Particles larger than 10 µm are deposited in nasal cavities, but particles smaller than 10 µm may not necessarily penetrate the lungs;
- No single method completely characterizes nasal sprays;
- Nasal cast is an interesting tool for intranasal drug deposition prediction;
- Imaging technics and Regions of Interest have to be standardized to ensure accuracy intranasal deposition measurement;
- Rapid nasal clearance and large delivery volume can affect deposition measurement accuracy;

Figure 3: Key considerations for In vitro – in vivo correlation of intranasal drug deposition

Table 1: Anatomical models reported in the literature (not exhaustive list). The illustration number refers to figure 2. "One-part" indicates that the model cannot be dismantled, (?): the items is not precisely described by the authors, MS: maxillary sinuses, MRI: magnetic resonance imaging, CT: computed tomography, ABS: acrylonitrile butadiene styrene, w/v: weight/volume, FESS: functional endoscopic sinus surgery.

Illustration number	Name of the model	Modelling process (material)	Geometry and particularities	Technical considerations
Simplified geometry				
1	Glass Sinus [24, 25]	Traditional manufacture (glass)	-one-part -tube with one simulated sinus	-transparent -reusable, washable
	Airway model [26]	Traditional manufacture (glass)	-one-part (?) -tube -coating with simulated mucus	-dismantlable model (?) -transparent (?) -different surface condition
2	Sinus/Nasal model [27]	Traditional manufacture (plastic)	-two parts -tube and syringe sinus	-dismantlable model -transparent, -reusable, washable -variable geometry,
	ENT anatomical model [28]	Traditional manufacture (plastic)	-two parts -one square fossae and one simulate MS	-dismantlable model -transparent, -reusable, washable
	PARI nasal cast [29-31]	Method not described (polyoxymethylene)	-eight parts -two plastic fossae with simulated turbinates (sagittal section) & glass vials for paranasal sinuses	-dismantlable model -reusable, washable, -variable geometry
	Tube Model [32]	Traditional manufacture (sapphire & sodium chloride crystal)	-one-part -tube and nasal valve "nozzle"	-dismantlable model -transparent, -reusable, washable -not designed for nasal deposition but for gas clearance quantification (infrared spectroscopy and radioactive krypton imaging)
3	Two-bottle model [33]	Traditional manufacture (plastic, soda bottle)	-two parts -one large bottle (500 ml) and one small (250 ml) + a plastic tube (neck)	-dismantlable model -transparent, -reusable, washable -variable geometry
Sophisticated but incomplete geometry				
	Nasal Airway models: Swift's replica, Guilmette1 replica, Guilmette2 replica [34-37]	Traditional manufacture by hand carving or by milling-machine (black acrylic)	-mean of 70 parts (axial sections) -built from MRI 53-year-old male, no paranasal sinuses, -applied for 30 male & 30 female geometries from individuals of African, American, Caucasian, Asian ethnical backgrounds	-dismantlable model -opaque model (?) -reusable, washable (?)
4	VIPER SLA (no illustration) [38]	Stereolithography on SLA 7000 or Viper Si2 machine printers (resin)	-one-part -built from MRI 53-year-old male -no paranasal sinuses	-transparent model -reusable, washable (?) -printer resolution VIPER > SLA
	Koken model (Koken Co. Ltd.,	Method not described	-two parts (sagittal section)	-dismantlable model -transparent model

	Tokyo, Japan) [39-44]	(silicone resin)	-geometry containing turbinates & olfactory region (no paranasal sinuses & no septum (?)	-reusable, washable -can be used with medical lubricant KY®, Sar-Gel®, KolorCut
	Bespak model [45-47]	Rapid prototyping by selective laser sintering technique (Nylon Duraform PA, USP level1 certified (medical grade)	-six parts (regional section) -one built MRI 3D file of 53-year-old male -another one from healthy female volunteer, (no paranasal sinuses),	-dismantlable model -opaque model -reusable, washable -can be sterilized -can be used with artificial mucus (75:25 ethanol:glycerol mixture)
5	Idealized nasal geometry [48]	Stereolithography (plastic)	-one-part -average geometry of the 3D-scans of Storey Bishoff models -MS are excluded, ethmoid reduced	-opaque model -reusable, washable (?) -second model in metal was built
	UPPER AIRWAY MODEL [32]	Stereolithography (material not described)	-one-part -built from CT-scan of healthy volunteer -no paranasal sinuses, ethmoid reduced	-opaque model -reusable and washable -not design for nasal deposition
	Nasal replica cast (derivated from VIPER) [49]	Stereolithography (resin polymerization)	-six parts (regional section) -identical as Viper model (MRI 3D file of 53-year-old male) -no paranasal sinuses, -dimensions available	-dismantlable model -transparent model -reusable, washable -SLA resin deteriorates over time, in contact with solvents and UV-radiation (Hughes, 2008)
	Nasal airway cast [50]	Stereolithography (polypropylene VeroClear)	-several parts (regional sections) -built from MRI 3D file of 53-year-old male -no paranasal sinuses -turbinate and olfactory region separated (cut-model)	-dismantlable model -transparent and rigid model -reusable, washable
9	Human nasal replica cast [51]	Stereolithography (material not described)	-two (or three?) parts (sagittal sections) -built from the MRI 3D file of 53-year-old male -no MS -sections designed for olfactory deposition measurement	-dismantlable model -opaque model -can be coated with a layer of 2% w/v mucin solution
	Nasal airway replica [52]	Stereolithography by fused deposition modeling (ABS)	-three parts (regional sections) -built from the MRI of 'subject 9′ of Golshahi et al. (2011) -no paranasal sinuses	-dismantlable model -reusable and washable -coated with 10% (w/v) Tween® 80, (a nonionic surfactant) to minimize particle bounce and re-entrainment -designed for nasal & lung deposition aerosol study
7	Nose sinus cast [53]	Stereolithography (polypropylene VeroClear)	-five (?) parts (regional sections) -built from MRI 3D file of 53-year-old male -no frontal and sphenoid sinus -three maxillary	-dismantlable model -transparent model -reusable, washable

			sinuses of varying sizes were fabricated	
	Nasal cast model from a 12-year-old subject [54]	Stereolithography (Durus white photopolymer)	-five parts (axial sections) -built from MRI 3D file of 12-year-old male -no maxillary sinuses, ethmoid reduced	-dismantlable model -opaque model -reusable, washable -can be coated with artificial mucus solution (~ 0.5–1 ml of a 4%w/v or 10%w/v mucin)
8	VCU nasal model [55]	Not described	-two parts -no paranasal sinuses	-dismantlable model -transparent model
6	Nasal cast replicas [56]	Stereolithography (Somos Watershed XC 11122)	-five parts (regional sections) -incomplete geometry (?) -ten models were built from anonymized CT-scans: five pediatrics female patients (7-14years); five from male and female adult (31-48years) -MS and sphenoid sinuses not described	-dismantlable model -transparent model -reusable, washable -face in translucent amber and flexible material
	Nasal cast (Boehringer Ingelheim Pharma GmbH & Co.) [57]	Stereolithography (material not described)	-five parts (regional section) -built from adult CT-scan -no paranasal sinuses, ethmoid reduced	-dismantlable model -opaque model -reusable, washable (?)
	Fisher & Paykel [58]	Stereolithography (silicone)	-one-part -built from CT-scan of a 44-year-old male -1.55 times scale -no paranasal sinuses	-transparent model -first designed for nasal airflow studies
	Realistic Nose-Throat (RDD model) [59]	provided in SolidWorks (EASM or EPRT) file formats for free downloading	-one-part -incomplete geometry (?) -5 years' geometry including nasal cavity, pharynx, and larynx, ~22.3 cm^3 -no indication about paranasal sinuses	-to be print by the user
Sophisticated and complete geometry				
	Nasosinus model [60]	With the assistance of Koken Co. Ltd. (silicone resin)	-three parts (?) -built from the nasal cavity of a cadaver -MS and ethmoid casts (built from adult man CT-scan) separately added to nasal cavity -with endoscopic sinus surgery -two ostium diameters (10mm & 3mm)	-dismantlable model -reusable, washable (?) -inferior and middle turbinates are in decongested state -deposition site of interest are coated with a transparent silicone grease sheet (carbon particle caption)
11	SAINT model (Sophia anatomical infant nose-throat) [61-63]	Stereolithography (liquid monomer resin, Stereocol®)	-one-part -built from 3D-scan of 9-month-old-caucasian female patient	-opaque model -reusable, washable -designed for lung deposition at first

	Models of infant nasal geometry [64]	Stereolithography (acrylic plastic)	-one-part -built from 3D-scan of infants (9 geometries, age ranged from 3 to 18 years-old) -including larynx and MS if presents.	-opaque model -reusable, washable (?) -test for geometry dimensions (CT-scan)
	prINT model (Premature Infant Nose Throat-Model) [65]	Stereolithography (photopolymer termed FullCure 720)	-one-part -built from the MRI scan of a preterm infant (32weeks)	-closed model -transparent and stiff -validated by CT-scan for its geometry
	Nasal cast model [66]	Stereolithography by fused deposition (acrylonitrile butadiene styrene (ABS)	-four parts (axial sections) -small model (healthy woman adult CT-scan) and large model (healthy man adult CT-scan)	-dismantlable model -opaque model -reusable, washable -ABS potential porosity
	Nasal replica (from plastinated head model) [67-68]	Stereolithography (non-porous resin)	-one-part -built from 3D-scan of Plastinated head model -exterior access to MS	-transparent model -closed model -reusable, washable
	NC2 Nasal cast 2 [69]	Stereolithography by fused deposition (ABS)	-four parts (axial sections) -based on CT-scan of plastinated head model -exterior access to MS	-dismantlable model -opaque model -reusable, washable -ABS potential porosity
10	APTAR/UoT/DTF model [70-72]	Stereolithography (epoxy plastic & silicone)	-four parts (axial sections) -based on CT-scan of plastinated head model	-dismantlable model -transparent -reusable and washable -nose in silicone material
13	Nasal airway models [73]	Stereolithography (Accura ClearVue, plastic meeting USP ClassVI (medical grade)	-five parts (regional section) -geometry including throat and oral cavity -built from CT scan of a toddler (2-year-old), a child (5-year-old) and an adult (50-year-old). -printed with high resolution for turbinate and olfactory regions, standard resolution for all others.	-dismantlable model -transparent and stiff -no deformity and congestion in airway passages.
	Hollow plastic model [74]	Stereolithography (Watershed® & rubber)	-two parts (?) -FESS geometry -built from post-surgery CT scan of a male subject with bilateral functional endoscopic sinus surgery (FESS) -anterior part of model in rubber	-dismantlable model (?) -transparent -reusable and washable
12	Nasal cavity phantoms	Method not described	-several parts (regional sections)	-dismantlable model -transparent model

	[75]	(skin-like soft material for face and nose)	-built from CT scans of 2,5,7,12,15, and 18-year-old children with normal/healthy nasal anatomy -exterior nose and flexible faces are available separately	-reusable, washable -can be customized for specifics applications
Human geometry				
	Head cadaver [76-80]	Cadaver specimens with no specific preparation	-one-part -complete anatomical structures -with or without endoscopic sinus surgery	-poor preservation -non-washable (?) -models with surgery available
14	Plastinated head model [81-85]	Plastination of head cadaver (polymers replaced water & lipids)	-one-part -complete anatomical structures -exterior access to MS -closed mouth	-reusable, washable -difficult to dry (?) -6 months to obtain one specimen -validated for nasal resistance (rhinomanometry), and geometry (CT-scan)