

An Enhanced Approach to Predicting Site Specific Inhaled Drug Deposition in Humans via In-Vitro and In-Silico Data Fusion

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Summary

The precision of aerosol deposition prediction in inhaled drug delivery is advancing through the integration of in-vitro experimentation and in-silico modelling. Building on the foundational work of Grill et al. [4], who demonstrated that the ability to predict dose deposition at the local site of action enables optimisation of aerosol and inhalation parameters, we present a novel data-fusion workflow linking physical aerosol testing with high-resolution human lung simulations in a time-resolved, geometry-matched manner. This collaboration between Crux Product Design Ltd. and Ebenbuild GmbH centres on high-dose, high-velocity aerosols delivered via the RS01 Dry Powder Inhaler (DPI), representative of a delivery device for the next generation of low-potency pulmonary therapies. Anatomically representative upper airway induction ports were derived from patient CT data, 3D printed, and integrated with a Malvern Spraytec system to enable real-time Particle Size Distribution (PSD) measurement during an inhalation event.

Tests were performed at 2 kPa and 6 kPa pressure drops using sieved Lactohale 300 lactose powder in a healthy airway model. Transient PSD and mass flow data were provided to Ebenbuild's human lung digital twin platform, Twinhale®, to simulate full-lung deposition outcomes using a patient-specific in silico model derived from the same CT dataset from which the anatomical airway induction port was created.

Results show that the anatomically representative upper airway geometry meaningfully influences the PSD and increases the Fine Particle Fraction (FPF), with downstream effects on predicted alveolar and regional lung deposition. In-silico analysis showed that higher flow rates, despite producing a smaller PSD, may increase conducting airway deposition due to greater particle inertia. By linking in-vitro and in-silico datasets, this study demonstrates an enhanced end-to-end methodology that could enable more effective and efficient optimisation of formulation, device and inhalation parameters to support future tuned and targeted inhalation therapies. It is plausible to consider that insights gained from this methodology could unlock new human-device interaction insights which could enable future novel drug-device design, including advanced design considerations for different disease states combined with disease severity and cohort. The approach may also enable future reductions in design and development timescales by potentially reducing experimental campaigns such as impactor testing, enabling a faster future pathway to human trials.

Key Message

By combining anatomically representative in-vitro testing with high fidelity, time-resolved aerosol measurements and in-silico lung simulations, this methodology enables precise prediction of site-specific drug deposition, unlocking a novel, data-synchronised in-vitro/in-silico workflow for tuning the device-dose design to target specific disease states/severities/cohorts, with reduced impactor testing, all enabling a faster pathway to market.

Introduction

Inhaled drug delivery is evolving with the advent of high dose therapies that require new levels of precision in aerosol characterisation and deposition modelling. Traditional in-vitro tests using standardised induction ports fail to capture the effects of anatomical variability on drug delivery.

To address this, a comprehensive case study using the RS01 DPI and an anatomically representative test rig, supported by digital human lung simulations was completed. This work - a collaboration between Crux Product Design Ltd. and Ebenbuild GmbH - demonstrates an enhanced approach to end-to-end

inhaler aerosol testing and human lung deposition prediction, combining experimental and Computational Modelling & Simulation (CM&S) tools.

A 3D printed and coated upper airway geometry was integrated with a laser diffraction measurement system to characterise post mouth and throat transient aerosol data as inputs to a patient-specific, physics-based digital twin of a human lung. This may in the future offer a cost-effective means for optimising drug-device combinations for targeted deposition, potentially enabling tailored respiratory treatments to individual therapy cases.

Experimental Methods

In-Vitro Characterisation of the Respirable Dose

This study evaluated the aerosolisation characteristics of a DPI (RS01) (Figure 1B) delivering 50 mg of sieved Lactohale 300 from size 3 hydroxypropyl methylcellulose (HPMC) capsules. The HPMC capsules were conditioned for at least 3 hours at 44% RH ($20\text{ }^{\circ}\text{C} \pm 0.5$) and weighed before and after testing to determine the emitted dose. Powder deposited within the inhaler was not quantified. Inhalation flow rates were selected to produce pressure drops of 2 kPa and 6 kPa across the inhaler, with inhalation durations set to achieve a total inhaled volume of 2 L. These test parameters were based on industry norms and inspiratory capacity approximately at the lower limit of normality for healthy subjects aged 9-81 years [1] [2]. Square-wave, constant flow was applied as per DPI testing to United States Pharmacopeia (USP <601>) [6].

To model the upper respiratory tract, a Computed Tomography (CT) derived 3D model of a healthy upper airway geometry was created and processed using Ansys SpaceClaim and SolidWorks. A rig incorporating the representative anatomy was designed to interface with Malvern's standard Spraytec laboratory laser diffraction system (950 mm bench, 300 mm lens) via the Inhalation Cell (STP2300). The representative anatomy included a mid-plane split to enable cleaning and reassembly. The geometries were 3D printed (Formlabs SLA, Grey Pro resin), and the internal surfaces were spray coated with a thin layer of a standard silicone oil (Figure 1A) to reduce re-entrainment of particles after wall impaction and more closely simulate the mucosal lining of the upper respiratory tract. Following application, excess silicone was allowed to drain, in line with common practice during in-vitro particle sizing [3].



Figure 1 – (A) Two-piece 3D printed anatomical throats with sagittal dissection, sealing cord and interfaces for Malvern Spraytec and RS01 DPI. Coating of internal surfaces with silicone oil to reduce re-entrainment of particles on wall impaction. (B) RS01 DPI device with Size 3 HPMC capsule inserted. (C) Test setup with 3D printed anatomical throat, Malvern Spraytec with Inhalation Cell, Copley TPK 2000-R Critical Flow Controller, Copley HCP5 Vacuum Pump and in-line TSI-4043 mass flow meter

The experimental setup comprised the RS01 DPI and the selected anatomical throat model connected to a Copley TPK 2000-R Critical Flow Controller and Copley HCP5 Vacuum Pump, with an in-line TSI-4043 mass flow meter. Capsules were loaded into the inhaler, pierced using the device by the operator (Figure 1B), and the device inserted into the mouthpiece interface of the upper airway model while attached to the Spraytec (Figure 1C). The Spraytec recorded transient aerosol Particle Size Distributions (PSD) at 1 kHz and normalised laser transmission data, used to calculate inferred mass flow rates at the tracheal outlet. Each scenario was tested in triplicate. Aerosol output was characterised both at the device outlet (device-emitted dose) and at the exit of the experimental throat geometries (respirable

dose) in separate tests. Where the device-emitted PSD was measured, the RS01 was connected directly to the Spraytec inhalation cell.

This setup enabled assessment of the respirable fraction exiting the upper airway, providing insight into how patient upper airway anatomy impacts the dose available for potential delivery to the lungs.

In-Silico Simulation of Respirable Dose

The measured post-throat PSDs and mass flow rates were used as transient inputs into Twinhale®, Ebenbuild's physics-based, in-silico simulation platform. Twinhale® simulates aerosol transport and deposition throughout the full respiratory tract, from the trachea to the alveolar sacs, using high-resolution, subject-specific lung models [4].

In this study, the lung model was derived from a CT scan of a healthy male subject (H04 from dataset [5]). The airway tree was extracted from the CT data to the extent permitted by image resolution and supplemented with artificially generated distal airways using a space-filling algorithm as described in Grill et al. [4]. The geometry was discretised into approximately 35,000 airway segments and 18,000 alveolar tissue units. Each simulation tracked 500,000 computational particles using Lagrangian particle dynamics, with drag, gravity, and buoyancy forces acting on them. All particles were modelled as spheres with a constant skeletal density of 1525 g/L; thus, volume and mass-based particle size distributions are considered equivalent (i.e., MMD = VMD).

While the in-vitro experiments were limited to the inhalation phase, the simulations represented a full breathing cycle: constant inhalation flow (aligned with the experimental condition), a 2-second breath-hold, and a 5-second slow exhalation, with exhaled volume matching the inhaled volume. The aerosol and particle properties (sizes, mass flow rate) were assigned based on the time-resolved in-vitro measurements (see Figure 2).

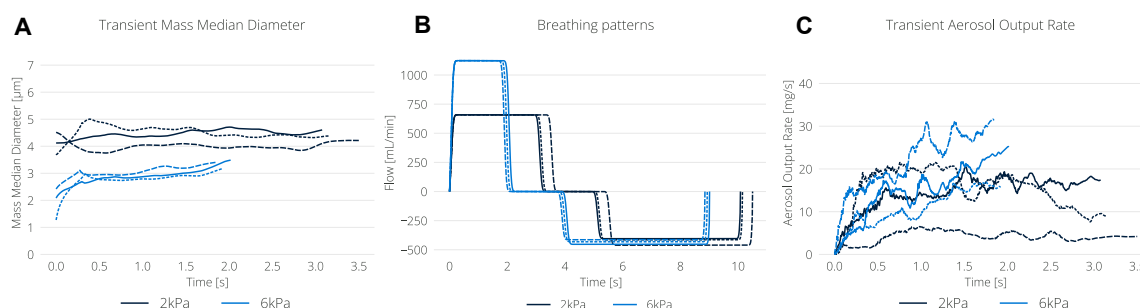


Figure 2 – (A) Transient mass median diameters from test data. (B) Breathing patterns used for Twinhale® simulations based on test conditions, including breath hold and exhalation with matched tidal volumes. (C) Transient Aerosol Output Rate inferred from emitted dose mass and laser transmission measured during physical tests. Each pressure drop condition (2 kPa and 6 kPa) is shown with the three experimental replicates.

Deposition fractions were normalised to the simulated input mass exiting the upper airway, allowing direct comparison of regional deposition patterns across conditions.

Results

Post-Throat Aerosol Characterisation

The inclusion of anatomically representative upper airway geometries significantly altered the PSD at the tracheal outlet. Across both pressure drops, tests using the airways produced smaller median diameters and higher Fine Particle Fractions (FPF) compared to tests without the airway, demonstrating the effect of impaction of inhaled aerosols within the upper airway. The magnitude of this shift of median

diameter increases with flow rate, with the 6kPa flow rate exhibiting a higher level of impaction in the throat (Figure 3).

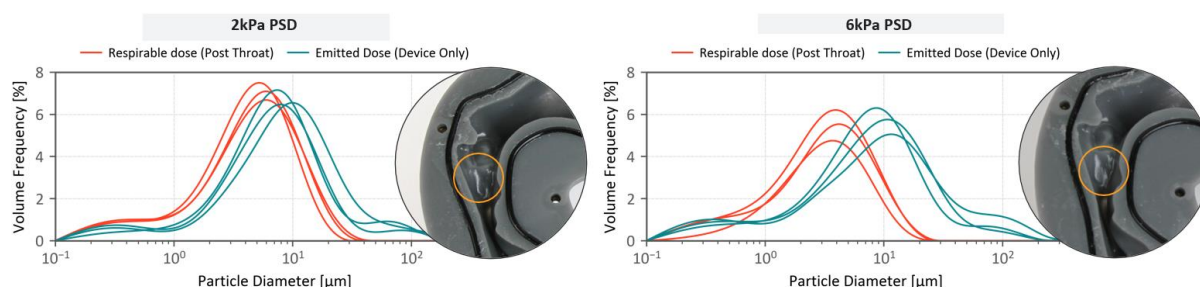


Figure 3 – Increased powder deposition in the throat during the 6 kPa (67.4 LPM) test compared to 2 kPa (39.4 LPM) test with total dose PSD (volume-weighted average PSD over full emission period) for each test, showing a pronounced increase in modal shift between device emitted dose and respirable dose tests at the higher flow rate.

In-Silico Lung Deposition Analysis

Figure 4 presents the results of the in-silico lung deposition study, evaluating aerosol transport and deposition for particles exiting the experimental throat model. The deposited mass within the mouth–throat region was not quantified experimentally. Therefore, all in-silico deposition results are expressed as fractions of the aerosol mass passing the tracheal inlet. Particle deposition was quantified regionally in terms of central versus peripheral lung compartments.

The distribution between central and peripheral region was surprisingly similar for the 2 kPa and 6 kPa pressure drop cases. This is somewhat unexpected, given that the 6 kPa case generated a notably smaller particle size distribution, which would typically favour deeper, peripheral deposition. A plausible explanation is that the higher inhalation flow rates at 6 kPa increases inertial impaction in the central airways, offsetting the expected increase in peripheral deposition from smaller particle sizes.

A functional deposition analysis was also performed, categorising regions by physiological role—specifically, conducting airways (thoracic) versus alveolar tissue. This extends the traditional central–peripheral distinction by providing a more physiologically accurate separation between conducting airways and alveolar regions. It accounts for the presence of alveoli in central parts of the lung and acknowledges that peripheral regions can still contain conducting airways.

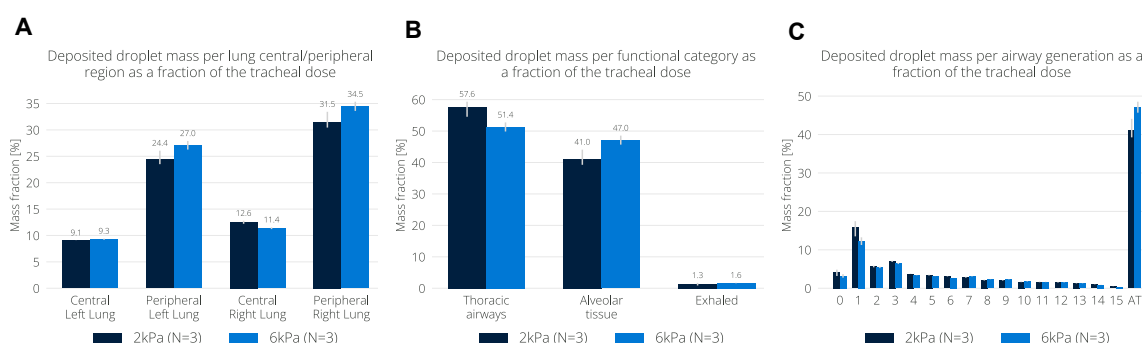


Figure 4 - (A) Deposited aerosol mass fractions across central and peripheral lung compartments (not shown: deposition in trachea and large bronchi and exhaled mass fraction) (B) Deposited aerosol mass fractions in thoracic airways, alveolar tissue, and exhaled fraction. (C) Deposited aerosol mass fractions by airway generation number (not shown: exhaled fraction). Bars show mean across the replicates. Vertical grey lines indicate the minimum and maximum values among the replicates.

The functional deposition analysis reveals a slightly more distinct difference between the two pressure conditions: The 6 kPa case shows approximately 6% more alveolar deposition compared to the 2 kPa case. For the 2 kPa case, which generally includes larger particles, substantial deposition likely occurs

at the carina and the first bronchial bifurcations. This is consistent with the airway generation-resolved deposition results, which show elevated deposition in generations 0 and 1 for the 2 kPa case.

Across all simulated scenarios, exhaled aerosol losses were minimal. This outcome aligns with expectations, given the breath-hold and slow exhalation conditions modelled in the simulations.

Discussion and Conclusion

This study presents an enhanced methodology for an end-to-end workflow to predict aerosol deposition from inhaler to alveoli. By integrating experimental throat models with patient-specific digital twin simulations, we demonstrate how particle size distribution, patient anatomy, and inhalation profile can influence lung targeting. The results confirmed that incorporating realistic airway geometries meaningfully altered post-throat particle size distributions and increased fine particle fractions, highlighting the role of upper airway impaction losses. Furthermore, the in-silico analysis revealed that although higher flow rates generate smaller particles—typically favouring peripheral deposition—the associated increase in particle inertia may limit the benefit through increased conducting airway deposition. These findings underscore the complex interplay between flow conditions, particle behaviour, and deposition outcomes. Such dynamics cannot be captured by simplified models, reinforcing the advantages of high-resolution, patient-representative simulations for accurate, cohort led predictions.

By combining advanced in-vitro methods with large, diverse cohorts of subject-specific lung models, simulation-powered datasets can now support increasingly precise predictions of deposition behaviour. This enables a powerful toolset for device design, formulation tuning, and regulatory strategy. Moreover, the full automation of the in-silico trial process enables the generation of comprehensive lung deposition data across large patient cohorts within a short timeframe of a few days. This efficiency offers a major advantage over traditional clinical trials, which are costly and time intensive. While in-silico trials cannot yet replace clinical studies in regulatory settings, they can already provide valuable early-stage insights during drug-device development, guiding design decisions long before clinical trials commence.

Future work may build on this foundation by incorporating measurement of case-specific respirable mass, improving the fidelity of upper airway deposition models through more representative simulation of mucus, incorporating realistic breath flow profiles and validating predictions across a wider range of patient anatomies and disease states. These advancements will further enhance the value of modern in silico tools for more effective and efficient optimisation of inhaled therapies in both preclinical development and clinical deployment.

References

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Response to the reviewers:

We would like to thank the reviewers for their careful evaluation of our manuscript and for their constructive comments. We have carefully addressed each point raised and revised the manuscript accordingly.

1. *Reference in the summary to Grill et al. has the wrong number citation*

Thank you for catching this. We have corrected the citation numbering. The reference to Grill et al. in the Summary now uses the correct citation number.

2. *Summary and Key Message – I'm not convinced of the novelty of this approach. It is not novel to combine in vitro measurement with in silico estimation of regional lung deposition. I encourage the authors to mention specific novel aspects of their approach in the summary and key message.*

We thank the reviewer for this valuable feedback. We agree that the combination of in-vitro and in-silico methods is in general not new. In this work, the novelty lies in the specific implementation of a time-resolved, geometry-matched data-fusion workflow, in which transient aerosol Particle Size Distribution (PSD) and mass-flow data measured in patient-specific physical upper-airway models are directly integrated into digital lung twins based on the same patient data. This approach enables high-resolution, flow-rate-dependent mapping of aerosol properties, providing more realistic in-silico deposition predictions than those obtained using static or otherwise aggregated aerosol properties.

To make this clearer, we have revised the Summary and Key Message sections to explicitly highlight these novel aspects.

3. *Summary and Introduction – the term 'low-potency' therapies is used. Is this the term the authors wish to use?*

We thank the reviewer for highlighting this point. Our intent was to convey that many emerging inhaled therapies require higher delivered doses compared with small-molecule bronchodilators and corticosteroids. We have revised the sentence to read: "Inhaled drug delivery is evolving with the advent of high dose therapies that require new levels of precision in aerosol characterisation and deposition modelling."

4. *Introduction, third paragraph: replace 'Spraytec' with 'laser diffraction'.*

Thank you for the suggestion. We have replaced "Spraytec" with "laser diffraction" in the third paragraph of the Introduction.

5. *Methods: it appears that constant flow rates, or square wave inhalation patterns, were used to test the DPIs. Please justify this choice, versus the use of simulated realistic inhalation manoeuvres, or mention as a limitation of the work.*

The Methods section has been amended to clarify that a square-wave flow profile was used in accordance with standard USP testing procedures. As noted in the Discussion and Conclusion sections, future work will extend this study by incorporating realistic inhalation profiles and validating predictions across a broader range of patient anatomies and disease states.

6. *Methods: CT is not capable of resolving lung geometry down to the smallest airway generations. Please revise to mention which airway generations in the lung model were derived from CT and what methods was used to estimate the dimensions of more distal lung generations.*

Thank you for this helpful comment. We have supplemented the description of the in-silico setup to clarify which airway generations were derived from CT data and how the dimensions of the more distal generations were estimated using algorithmic reconstruction methods.

7. *Results: Figures 3 and 4 appear blurry and thus are hard to read*

Thank you for pointing this out. We have replaced Figures 3 and 4 with higher-resolution versions to improve readability.

8. *Results: Please present the extrathoracic deposition fraction. From Figure 3, the VMD of the aerosol exiting the device is ~10 micrometers, therefore high deposition in the mouth and throat is expected. This, then will be a major determinant of the lung dose.*

Thank you for raising this important point. Determination of the deposited mass within the mouth–throat region was not performed in this study, as direct quantification was experimentally challenging within the anatomical model. Consequently, the in-silico simulations were normalised to the tracheal dose, and the reported deposition fractions represent regions distal to the trachea. We have clarified this point in the Results section.

9. *Discussion: I'm not convinced that it has been demonstrated in this work that PSD, patient anatomy and inhalation profile are key determinants of lung targeting. Only one powder was tested, thus there is limited variation of PSD exiting the device, only one patient anatomy was tested, and constant flow rates were used instead of more realistic inhalation profiles. Please revise this statement in the discussion.*

Thank you for this helpful comment. We have revised the statement in the Discussion. The text now clarifies that the results illustrate potential influences of PSD, anatomy, and inhalation profile on lung targeting, rather than demonstrating them conclusively.

10. *Please revise throughout to generalize statements that currently focus on one commercial product. For example the concluding sentence reads: These advancements will further enhance Twinhale®'s value as a tool for more effective and efficient optimisation of inhaled therapies in both preclinical development and clinical deployment. This could be revised to comment on in silico models in general, rather than one specific commercial offering.*

Thank you for this suggestion. We have revised the manuscript to generalize statements that previously referred specifically to Twinhale, where applicable.