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*In-silico* quantification of intersubject variability on aerosol deposition in the oral airway

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**Abstract:** A single paragraph of about 200 words maximum. For research articles, abstracts should give a pertinent overview of the work. We strongly encourage authors to use the following style of structured abstracts, but without headings: (1) Background: Place the question addressed in a broad context and highlight the purpose of the study; (2) Methods: briefly describe the main methods or treatments applied; (3) Results: summarize the article’s main findings; (4) Conclusions: indicate the main conclusions or interpretations. The abstract should be an objective representation of the article and it must not contain results that are not presented and substantiated in the main text and should not exaggerate the main conclusions.

**Keywords:** Computational fluid dynamics (CFD); Inertial impaction; Laryngeal particle deposition; Oropharyngeal deposition

1. Introduction

The extrathoracic upper airway acts as the first line of defense to prevent inhaled toxicants from reaching the lungs but also as a barrier to the delivery of inhaled drugs. The proportion of aerosol deposited in the oral cavity and throat depends on the flow field and the size of inhaled particles [1]. The intricate anatomy of the upper airway produces complex flow patterns and particle trajectories and is thus also an important factor affecting aerosol deposition in the human respiratory system [2]. Several studies have shown that deposition in the throat is a major source of variability in lung deposition [3-5].

Aerosol deposition in the oral airway has been studied both computationally [2, 6] andexperimentally [7-9]. Previous *in vitro* work with steady flow rates presented empirical correlations for predicting oral deposition [10, 11]. However, previous studies mostly evaluated total upper airway deposition and have not distinguished deposition in the oropharyngeal and laryngeal region. This distinction could be important. For instance, laryngeal deposition of inhaled corticosteroids (ICS), a mainstay in the treatment of chronic reactive airway disease, elicits local side effects, including dysphonia [12, 13], that could be minimized with optimized flow rate for a specific upper airway anatomy.

Understanding the mechanics of particle deposition in the upper airway is useful to design new inhalation therapies for respiratory diseases [14]. It also helps estimating exposure risks to inhaled toxicants [15] or airborne transmission of SARS-CoV-2 laden droplets [16]. Computational fluid particle dynamics (CFPD) has been used as a reliable method to predict airflow and particle deposition in the human airway [17-19]. In this study, we have undertaken CFPD studies to characterize deposition of micrometer-sized particles (1-30 µm) at subject-specific inhalation flow rate in realistic geometries of the upper airway reconstructed from computed tomography (CT) scans of 11 individuals. Intersubject variability in shape and volume of the upper airway is expected to have a significant impact on the deposition of inhaled aerosols filtered by this region.

2. Materials and Methods

1. *CT database and subject characteristics*

The 3D upper airway models were based upon CT images from seven healthy male subjects and four male subjects with mild-to-moderate chronic obstructive pulmonary disease (COPD). Images were previously obtained on a GE Light Speed Discovery CT750 and acquired as part of a Bioengineering Research Partnership (NIH R01-HL-073598). Images of the head and torso were obtained in the supine position during a breath hold at a lung volume of 1 liter above functional residual capacity (FRC). CT scans were obtained with the mouthpiece positioned in the patient's mouth. The field of view was 36 × 36 × 48 cm in the x, y and z dimensions (with the z axis in the cranial to caudal direction). The matrix size was 512 × 512 x 960, generating voxel dimensions of 0.7 × 0.7 × 0.5 mm. Acquisition and use of these images were approved by the Institutional Review Boards of the University of Washington, Seattle and the University of California, San Diego, respectively. Anthropologic and lung function data of all subjects are presented in **Table 1**. Geometric data of all subjects are presented in **Table A1** (see appendix A) along with the 3D reconstructed geometry of the eleven subjects (**Figure A1**).

**Table 1**: Anthropometric data.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Subject ID** | **Age, Yr** | **Weight, kg** | **Height, cm** | **BMI** | **Health Status** | **FEV1, %pred** | **FEV1/FVC** |
| H1 | 35 | 68.2 | 170 | 23.5 | Healthy | 113 | 0.88 |
| H2 | 52 | 96.8 | 165 | 35.5 | Healthy | 117 | 0.79 |
| H3 | 47 | 88.6 | 183 | 26.5 | Healthy | 85 | 0.74 |
| H4 | 26 | 91.8 | 183 | 27.5 | Healthy | 94 | 0.80 |
| H5 | 34 | 100 | 193 | 26.8 | Healthy | 104 | 0.84 |
| H6 | 21 | 54.1 | 168 | 19.2 | Healthy | 89 | 0.73 |
| H7 | 21 | 63.6 | 173 | 21.3 | Healthy | 95 | 0.81 |
| COPD1 | 57 | 70 | 164 | 26.1 | COPD | 60 | 0.56 |
| COPD2 | 55 | 65.9 | 178 | 20.8 | COPD | 56 | 0.48 |
| COPD3 | 45 | 83.2 | 180 | 25.6 | COPD | 69 | 0.67 |
| COPD4 | 54 | 83.6 | 187 | 24 | COPD | 58 | 0.52 |
| FEV1, forced expiratory volume in 1s; FVC, forced vital capacity, %pred: % predicted. | | | | | | | |

1. *Reconstruction of human upper airway geometries*

CT scans in DICOM format were used to create three-dimensional (3D) realistic models of the oral airway anatomy using Mimics 23.0 (Materialise Inc, Leuven, Belgium). Briefly, by setting a previously identified optimal threshold between -1024 and -300 Hounsfield Units (HU) [20, 21], the 3D anatomy of the oral cavity including mouth, oropharyngeal region, larynx, vocal cord, and upper tracheal sections were selected as the region of interest (ROI). The nasal cavity and paranasal sinuses were excluded. The oral airway geometries were exported in stereolithography (Stl) format to ICEM-CFD 21.0 (ANSYS 2021 R2, Inc, Canonsburg, Pennsylvania), where planar inlet and outlet surfaces were defined. All geometries were oriented with the oral cavity floor parallel to the Z-axis (**Figure 1**). Planar surfaces of the posterior-mouth, oropharynx, area of larynx and trachea were created for simulated airflow analysis. A 245 mm-long tube was added to the mouthDiagram

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**Figure 1.** Definition of the subregions of the upper airway (A) CT scan of the oral airway of subject H1. (B) Reconstructed 3D model. The mouth is the oral cavity where extends from the back of the teeth to the uvula. The oropharynx region is the cavity from the back of the uvula to the tip of epiglottis. The larynx from the tip of the epiglottis to below the vocal cords and top of the trachea.

1. *Computational Fluid-Particle Dynamics (CFPD) Simulations*

*2.3.1. Flow simulations*

CFPD simulations were performed by solving Navier-Stokes equations with Lagrangian particle tracking. Particle number density was sufficiently low so that particle motion did not affect airflow. The Navier-Stokes equation for incompressible flow is

=- (1)

where is the fluid velocity vector,   kg/m3 is the fluid density,   kg/ (m.s) is the dynamic viscosity of the fluid, is pressure and is time. To solve the Navier-Stokes equation, mesh independence analysis has been performed and all geometries were meshed in ICEM-CFD 21.0 with approximately twelve million tetrahedral elements and eight prism layers with total prism zone thickness of 0.2 mm. Prism mesh was generated near the boundary surface to ensure capturing of the rapid near wall changes in air velocity and particle deposition profile. Inhalation rates representative of resting and fast breathing were considered. Mesh quality was checked by considering the quality criteria of greater than 0.1 to ensure that the mesh had no distorted elements [22].

Experimental evidence suggests that airflow in the extrathoracic airway is laminar at resting breathing rates [23, 24]. Thus, for slow-breathing condition (18 L/min), equation (1) was solved in the steady-state laminar flow regime by employing Fluent 2021 R2 (ANSYS, Inc., Canonsburg, PA) and choosing SIMPLEC algorithm with pressure-velocity coupling as the solver and second-order upwind discretization method. Steady-state flow simulations were obtained by imposing boundary conditions on the inlet mass flowrate of the air and pressure outlet boundary conditions applied at the outlet boundary. To ensure numerically accurate flow simulation results, the Shear-Stress Transport κ-omega turbulent model (SST κ-ω) was employed to simulate the inspiratory and expiratory airflow for fast-breathing condition (45 L/min). The turbulence length scale was considered 0.001 m, and turbulent intensity was assumed to be 5% at the inlet and outlet [25, 26].

The following boundary conditions for the flow were applied: (1) zero velocity at the walls (no-slip boundary condition), (2) inlet mass flow at the mouthpiece’s inlet to set airflow of 18 and 45 L/min, and (3) zero pressure at the outlet.

*2.3.2 Particle transport simulations*

Forces affecting particles for the size range used in this study included sphere drag force and gravity (consistent with experimental orientation), allowing modeling of deposition mechanisms of inertial impaction and sedimentation. The equation of motion governing the trajectory of a particle is

(2)

where is the particle velocity, is the density of the particle, is the drag force per unit particle mass and

= (3)

where is the particle diameter, Re, the Reynolds number and is the drag coefficient.

Lagrangian-based Discrete Phase Model (DPM) was used to predict particle deposition in the eleven anatomically realistic models. Deposition of particles with mass median aerodynamic diameters (MMAD) of 1-30 µm were investigated during resting and fast-breathing conditions. Particles were considered inert particles with spherical shape and density of 1000 kg/m³ so that the particle diameter corresponds to the aerodynamic diameter. Particles were released from the inlet planar surface of the tube. As the tube and mouthpiece were not in the region of interest, the boundary condition “reflect” was applied for running DPM simulation. Boundary condition “trap” was considered for mouth cavity, oropharyngeal, laryngeal, and tracheal region. The trajectories of 10,000 particles were simulated for each particle size and each inhalation flowrate, allowing for particle number-independent predictions. Increasing to 100,000 particles changed predicted deposition by less than 0.38%.

The percentages of inhaled particles depositing in each anatomical region (**Figure 1**) was quantified. For instance, particle deposition in the laryngeal region was defined as 100\*(NL/NI), where NI is the total number of particles inhaled and NL is the number of particles deposited on the surface region mapped as laryngeal region on the airway model. Similarly, the total deposition efficiency was defined as 100\*(NT/NI), where NT is the total number of particles deposited in all airway regions combined.

1. *Comparison of subject-specific in silico predictions with experimental results*

Whole-lung deposition predictions were obtained for breathing parameters and functional residual capacity (FRC) that matched on a subject-by-subject basis those measured in 7 healthy subjects during controlled breathing of aerosols [27]. In these experiments, subjects were asked to target a tidal volume of 1000 mL of particle-laden air (1 and 2.9 µm) at constant inhalation flow rates of 18 and 45 L/min. Actual tidal volumes and flow rates are listed in **Table 2** along with each subject FRC.

**Table 2.** Patient-specific flowrates in healthy subjects.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Subject** | **H1** | **H2** | **H3** | **H4** | **H5** | **H6** | **H7** |
|  |  | **FRC (L)** | 3.26 | 3.38 | 3.44 | 3.51 | 2.67 | 3.43 | 3.31 |
| **dp = 1 µm** | **slow**  **breathing** | **Qin (L/min)** | 19.26 | 19.08 | 19.86 | 17.70 | 18.72 | 17.64 | 18.18 |
| **Qex (L/min)** | 20.94 | 19.32 | 20.10 | 18.24 | 18.66 | 17.82 | 17.76 |
| **TV (L)** | 1.116 | 1.073 | 1.101 | 0.979 | 1.041 | 0.984 | 1.009 |
| **fast**  **breathing** | **Qin (L/min)** | 43.56 | 41.40 | 41.94 | 39.18 | 40.56 | 42.90 | 41.16 |
| **Qex (L/min)** | 45.30 | 40.02 | 41.64 | 40.50 | 42.18 | 40.02 | 40.74 |
| **TV (L)** | 1.127 | 1.019 | 1.038 | 1.012 | 1.043 | 0.924 | 0.923 |
| **dp = 2.9µm** | **slow**  **breathing** | **Qin (L/min)** | 21.66 | 19.38 | 17.70 | 17.16 | 18.66 | 18.18 | 18.30 |
| **Qex (L/min)** | 23.16 | 18.96 | 17.94 | 17.70 | 18.00 | 19.26 | 19.14 |
| **TV (L)** | 1.254 | 1.064 | 0.987 | 0.972 | 1.023 | 1.043 | 1.249 |
| **fast**  **breathing** | **Qin (L/min)** | 45.48 | 44.58 | 39.96 | 37.62 | 40.32 | 40.08 | 40.26 |
| **Qex (L/min)** | 49.02 | 44.22 | 39.60 | 41.40 | 40.14 | 39.24 | 41.64 |
| **TV (L)** | 1.164 | 1.135 | 0.994 | 0.993 | 1.035 | 0.900 | 0.901 |
| dp: particle diameter, Q: flow rate, in: inspiration, ex: expiration, FRC: functional residual capacity, TV: tidal volume | | | | | | | | | |

Overall retained fraction was calculated as the sum of deposition fraction in the oral cavity and retained fraction in the intrathoracic lung. Intrathoracic deposition was estimated with the latest version of the Multiple-Path Particle Dosimetry (MPPD) model that includes a mechanistically based model component for alveolar mixing of particles and that accounts for multiple breaths of aerosol intake [28]. Deposition in the oral cavity was obtained from CFD simulations as described above. Deposition in the oral cavity was also obtained from a semi-empirical formula [29]:

(4)

where *da* is the aerodynamic diameter expressed in μm and *Q* the overall volumetric flow rate expressed in ml/s.

Mass of particles injected in the MPPD model was set as (1 - ηoral) x *Cinh* where *Cinh* is the inhaled particle concentration. Deposition in the oral cavity during exhalation was based on the mass of particles exiting MPPD (*ηoral Cexh.MPPD*).

1. *Statistical Analysis*

The curve of best fit of in-silico predictions of aerosol deposition in the oral cavity was calculated by fitting and to a sigmoidal function , where is the same impaction parameter used in the Stahlhofen equation (Eq. 4). , , and the percentage of variance explained () by the curve of best fit were compared to the parameters adapted in the Stahlhofen equation.

To compare in-silico predictions with experimental data, a one-way ANOVA test for correlated samples was used with the Tukey Multiple Comparison post hoc test. The paired t test was used to compare in-silico predictions of oral deposition between inspiration and expiration. Significant differences were accepted at the p < 0.05 level.

3. Results

1. *Total oral deposition efficiency*

**Figure 2** illustrates oral deposition efficiency as a function of the commonly used impaction parameter da2Q, where da (µm) is aerodynamic diameter and Q (L/min) is the flow rate. In agreement with previous *in-vitro* and *in-silico* studies [8, 9, 18, 30], the high correlation (**Fig. 2**, best fit, R2 = 86.02%) between oral deposition and the impaction parameter da2Q shows that inertial impaction is the dominant deposition mechanism in the upper airway. Total oral deposition of inhaled particles increased with increasing particle size and inhalation flowrate. For instance, in subject H1 and for a flow rate of 18 L/min, oral deposition increases from 0.26% for 1µm particles (black + symbol at da2Q = 18 µm2L/min, Fig. 2) to 1.49% for 5µm particles (black + symbol at da2Q = 450 µm2L/min) and to 20.71% for 10µm particles (black + symbol at da2Q = 1800 µm2L/min). In other words, 99.74% of 1µm particles, 98.51% of 5µm particles and 79.29% of 10µm particles traversed the airway model and were delivered to the intrathoracic region of the lung. Increasing the inhalation rate caused a substantial increase in the percentage of particles deposited in the upper airway. Simulations for a 45 L/min inhalation rate predicted 1.21% of 1µm particles (blue + symbol at da2Q = 45 µm2L/min, Fig. 2) and 76.78% of 10µm particles (blue + symbol at da2Q = 4500 µm2L/min depositing in the upper airway model, respectively. While most of 1 µm particles were delivered to the trachea at both flow rates, <25% of inhaled 10 µm particles were delivered to the intrathoracic lungs at the higher flow rate compared to ~80% at the low flow rate. Despite larger intrasubject variability in oral deposition, similar trends were observed for all subjects (**Figure 2**). For instance, deposition of 3 µm aerosols ranged from 0.51% to 11.59% (median = 0.97%) between subjects at 18L/min (i.e., for da2Q=162 µm2L/min) and from 0.84% to 52.32% (median 6.24%) at 45L/min (i.e., for da2Q = 405 µm2L/min). There was no significant difference in oral airway deposition between healthy and mild-to-moderate COPD subjects.

**Scatter chart

Description automatically generatedFigure 2.** Oral airway deposition vs. inertial impaction parameter. Gray symbols and black symbols represent deposition fraction at inhalation flowrate 18 (L/min) and 45 (L/min), respectively. Empirical prediction is based on Stahlhofen equation (Eq. 4).

Predictions of oral deposition in both healthy and COPD cohorts shows a good agreement with the empirical curve previously obtained by Stahlhofen et al. [29] from controlled *in vivo* experiments (red curve, **Figure 2**). Equation of best fit to patient-specific CFD data was 1 - (1/(a\*x^b+1)), where a = 6.73 e-08 (-7.33 e-09, 1.42e-07), b = 1.65 (1.55, 1.74) (R2best ﬁt = 86.02%).

1. *Effect of particle impaction on regional deposition*

Regional deposition fractions were computed as a function of da2Q for all the subregions of the upper airway as defined in **Figure 1**. There was a significant intersubject variability in the distribution of deposited particles among all three subregions of the upper airway (**Figure 3A-C**). As for total deposition, deposition in the mouth cavity increased with increasing particle size and increasing flow rate (**Figure 3A**). Deposition in both the oropharyngeal and the laryngeal region followed a bell shape, with the maximum deposition varying largely between subjects: maximum deposition occurred for an impaction parameter ranging between 103 and 1.2x104 in the oropharyngeal region (**Figure 3B**) and between 400 and 5000 in the laryngeal region (**Figure 3C**). Regional deposition as a function of particle diameter can be found in Appendix B (**Figure B1**).

Chart, histogram

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**Figure 3**. (A) Mouth cavity deposition fraction vs. impaction parameter. Dashed line is representing the results at inhalation flowrate 18 (L/min) and continues line is showing the results at 45 (L/min) (B) Oropharyngeal deposition fraction vs. impaction parameter (C) Laryngeal deposition fraction vs. impaction parameter. (D) Overall oral deposition

**Figure 4** shows the spatial distribution of deposited particles following inhalation of 3 µm aerosol in two subjects with highly different upper airway shape. (Similar data are shown in the appendix for 5 and 10 µm particles in the same subjects (**Figures B2 and B3**)). Hotspots of deposited particles were found on the posterior oropharyngeal wall (**Figure 4B, lower panel**), and for particles that deposited in the laryngeal region, at the level of the larynx and vocal cord (**Figure 4B, upper panel**). While in subject H6, most of deposition occurred in the oropharyngeal region (**Figure 4B, lower panel**), deposition is subject H5 was mainly located in the laryngeal region (**Figure 4D, upper panel**), highlighting the large variability in deposition patterns between subjects.

Figure 4: Spatial distribution of deposited 3 µm particles inhaled at 45 L/min in two subjects with largely different upper airway anatomy. (A) Isometric view (B) Back view(C) Front view (D) Side view. Upper panel: subject H5; Lower panel: subject H6.

1. *Comparison of inspiratory and expiratory particle deposition in the upper airway*

The effect of flow direction (inspiratory versus expiratory flow) on oral deposition was also investigated. Distribution of 1, 3 and 5 µm deposited particles at 18 L/min and 45 L/min breathing conditions among subregions of the subject-specific oral airway is shown for both inhalation and exhalation in **Figure 5**. These CFD results do not show any consistent trend when deposition occuring during inhalation is compared to that during expiration, with some subject showing higher deposition during inhalation, others showing higher deposition during exhalation and a third group showing similar values between inspiration and expiration. As a result, there was no significant difference in oral deposition between inspiration and expiration in this group of subjects. This was also true for subregion deposition except for particles ≥5 µm (**Figure 5F**) where most particles deposited in the laryngeal region during expiration, leaving few particles to travel and potentially deposit in the oropharynx and mouth cavity.

Figure 5: Comparison of deposition occuring during inhalation (IN) and exhalation (EX) in the different subregions of the oral airway. (A) 1µm particles at 18 L/min. (B) 1µm particles at 45 L/min. (C) 3µm particles at 18 L/min. (D) 3µm particles at 45 L/min. (E) 5µm particles at 18 L/min. (F) 5µm particles at 45 L/min.

1. *Comparison of in silico predictions with in vivo experimental data*

Whole-lung deposition predictions were obtained by coupling subject-specific CFD results with MPPD predictions as described in section 2.4. **Figure 6A** displays these predictions against experimental data obtained by Darquenne et al. [27]. Experimental data are displayed as deposition measured over five consecutive breaths (mean ± standard deviation (SD)). Whole-lung deposition were also obtained by coupling predictions from Equation 4 with MPPD (**Figure 6B**). Regression line between in-silico predictions (y) and experimental data (x) were y = 0.931x + 0.030; R2= 61%, for MPPD/CFD results (**Figure 6A**, dashed line) and y = 0.995x - 0.028; R2 = 73%, for MPPD/empirical results (**Figure 6B**). **Figure 7** compares whole-lung deposition predictions for the seven subjects grouped together for both the MPPD/CFD and MPPD/empirical cases and the experimental data. Data are presented as median (minimum, maximum).

**Figure 6**. Comparison of *in-silico* predictions with experimental data. Upper airway deposition was calculated from Eq 4 (panel A) or predicted by CFD (panel B). Retained fraction in the intrathoracic lung was obtained from the Multiple-Path Particle Model (MPPD) in both cases. See text for details.

**Figure 7.** Comparison of retained fraction predicted by the MPPD/empirical and MPPD/CFD models with measurements of Darquenne et al. (2016). In-silico predictions were obtained for subject-specific breathing conditions matching the experiments. Data are shown as the median (minimum, maximum) of the 7 healthy subjects. \*: significantly different from experiments (p<0.02).

4. Discussion

*4.1 Intersubject variability in deposition in the oral airway*

Oral deposition of particles in the aerodynamic size range of 1–30 µm was numerically predicted in eleven replicas of oral airways of adults. For any given combinations of particle size and inhaled flow rate, large scatter was observed between subjects. For example, deposition ranged from 0-18%, 1-56% and 2-85% at an impact parameter of ~200, 400 and 1000 µm2 L/min, respectively (Figure 2). These data compare well with in-vitro measurements obtained in nine replicas of oral airways where deposition ranged from 0-30%, 0-60% and 5-95% at an impact parameter of 200, 400 and 1000 µ2 L/min, respectively [9]. Similar scatter in oral deposition between subjects was also observed by Grgic et al. in a separate *in-vitro* study [8].

Add a graph with data plotted against Stk\*Re like in figure 4 of Golshahi et al (2013) + discuss reduction in data scattering?

Data from *in-viv*o studies have also reported large intersubject variability [31-37]. Using these *in-vivo* data, Stahlhofen et al. derived a semi-empirical equation based on particle size and breathing pattern characterized by the impaction parameter (Eq. 4). We developed a similar correlation based on our *in-silico* predictions (Figure 2). Despite pronounced intersubject variability in deposition predictions, the sigmoidal curve of in-silico oral deposition versus impaction parameter remains statistically indifferent from the experimental curve predicted by the Stahlhofen equation, which alone explains the experimental data well with a best fit R2 value of 85.98%. This compares to a R2 of 86.02% for the sigmoidal curve based on *in-silico* predictions.

Fewer studies have looked at regional deposition within the oral extrathoracic airway but it is often assumed that most deposition occurs at the level of sudden constrictions present in the oropharyngeal and laryngeal regions [29, 34]. Our data suggests this to be the case for impaction parameters up to ~1000-2000 µm2 L/min. However, for larger values of the impaction parameter, deposition in the mouth also becomes significant (Figure 3).

**These highlighted paragraphs are place holder. Will be updated/modified with comparison for similar impaction paramters between this study and Grgic study**:

Comparison of the regional deposition obtained in this *in-silico* study with *in vitro* data from the literature (Grgic et al. (2004)) demonstrated that considerable variations in the morphological geometries caused quite different deposition quantities. In general, our numerical results showed that multiple factors such as particle size, flow rate, flow direction and overall anatomical features affect deposition efficiency. It was in consistent with Grgic et al. and the reason for disagreement in sub-zonal deposition is the difference in amount of inhalation flow rate (18 L/min vs 30 L/min).

High levels of deposition of larger particles in the mouth and upper airway has also been demonstrated in Figure 4 and Figures A4-A5. The use of defined regions to compare the efficiency of deposited inhaled particles in the specific regions of the airway is complicated by hotspots of increased deposition at particular segments of the model. These areas of increased deposition can be easily seen upon visualization of the particle deposition patterns (**Figure 4**). In particular, our data demonstrates a hotspot of particle deposition at the back of the oropharynx and proximal larynx (**Figures A4-A5**). In addition, non-uniform distribution of deposited particles implies that the dose at the most vulnerable structures of the larynx may be different from the regional average. Although it is possible to define smaller regions on the model surface for more localized predictions, such approach may lead to less accurate results because predictions for smaller regions are more sensitive to computational methods (e.g., mesh size) and also require a larger sample size of airways for reliable statistics. When comparing the 3 m particle size and the 10 m particle size of inhaled particles at the inhalation rate of 45 L/min, subject H5 demonstrated that 10 m particles had significantly less laryngeal deposition than 3 m particles (2.1% and 45.74% respectively) (**Figures 4, A5**).

*4.2 Differences in regional deposition between inhalation and exhalation*

Most of the studies on upper airway deposition during mouth breathing have focused on the inspiratory phase, e.g [2, 5, 8, 9, 30], with very few looking at deposition during expiration [7, 38]. This is mainly because inhalation drug therapies are designed to maximize deposition in the lungs prior to exhalation. Indeed, when using a dry powder inhaler (DPI) or a pressurized metered-dose inhaler (pMDI), proper drug inhalation techniques call for a slow inhalation followed by a breath hold to allow for particles to settle in the airspaces, minimizing the number of particles being exhaled [39]. However, poor inhalation technique can result in a significant fraction of exhaled aerosol. Also, drugs administered with nebulizers do not typically include an end-inspiratory breath- hold, which can result in significant exhaled fractions.

Due to the paucity of available data for exhalation mode, some authors have suggested that deposition in the oral cavity during exhalation could be neglected [29] while others have proposed that inspiratory and expiratory deposition efficiency in the oral cavity should be considered to be equal [7, 40]. Our data suggest the latter to be a reasonable hypothesis, at least for micron-sized particles. Indeed, there was no significant difference between inspiratory and expiratory deposition in the oral cavity for this size range (Figure 5). This is also supported by data from Verbanck et al [38], albeit performed in a single oral airway geometry, that showed almost identical oral deposition curves for inspiration and expiration for experiments performed with 3 and 6 µm particles.

In terms of regional deposition, intrasubject differences were observed when airflow direction was reversed. While during inspiration, hot spots were mainly found in the oropharyngeal region and also in the mouth for the largest partciles (10 µm and up), high deposition was preferentially found in the laryngeal region during expiration. When averaged over all subjects, these differences were statistically significant for particles ≥ 5 µm. This may be of minimal clinical relevance for the largest particles (> 20 µm) that tend to have high intrathoracic deposition rates, leaving only a negligible particle fraction, if any, to be exhaled. In contrast, different deposition patterns may be of importance when assessing side effects of an inhaled drug with particle size distribution in the range of 1-10 µm, typical of most pharmaceutical aerosols. For example, the delivery of inhaled corticosteroids (ICS) is highly effective at controlling the inflammatory component of chronic airway disease such as in asthma and COPD, while limiting systemic toxicities [41]. However, the efficacy of ICS is highly dependent upon its ability to bypass the upper airway. High extrathoracic deposition not only limits the amount of drug that can reach the lungs but can also result in unwanted local side effects. Indeed, repeated deposition of in the larynx can cause a wide variety of clinical side effects including hoarseness, sore and/or dry throat, dysphonia, and candidiasis [12, 42].

*4.3 Comparison of whole-lung deposition with experiments.*

In an attempt to reproduce aerosol exposure studies performed in the same subjects from which the upper airways geometries were obtained, we predicted deposition for breathing maneuvers similar to that used in the experiments [27]. To do so, we coupled our predictions of oral deposition to intrathoracic deposition obtained with an improved MPPD model [28] that also accounted for subject-specific lung volumes and subject-specific inhalation and exhalation flow rates. (Table 2). As the MPPD model assumes a uniform ventilation among the different regions of the lung, we limited our comparison to whole-lung deposition data obtained in healthy subjects for which, unlike in COPD subjects, a uniform ventilation distribution is a reasonable assumption. Comparison of our MPPD/CFD predictions with experimental values shows relatively good agreement (Figure 6A) as did the comparison between experimental data and MPPD predictions coupled with Stahlhofen equation for oral deposition (MPPD/empirical predictions, Figure 6B). As the Stahlhofen equation only incorporates particle size and flow rate characteristics, less scatter was observed in the MPPD/empirical predictions than in the MPPD/CFD data, the latter also reflecting the effect of upper airway geometry on oral deposition (Figure 7).

There are a few limitations worth noting that could have affected our predictions. First, CFD simulations were performed in upper airway geometries with rigid walls that were based on CT images obtained at the end of a one-liter inspiration. Thus, the effect of any variation in the upper airway geometry that occurs during tidal breathing even in healthy subjects with no upper airway pathology [43, 44] was neglected. Second, as CT imaging and aerosol studies were performed at two different occasions, it is highly probable that the position of the tongue within the oral cavity changed between the sessions. Furthermore, the position of the tongue was not controlled for during the aerosol studies and its position may well have moved during the experiment. This would affect deposition predictions in the oral cavity. Indeed, previous studies have shown that the position of the tongue significantly affect the delivery of aerosol in the trachea, highlighting the high intrasubject variability in aerosol delivery to the lungs [45-47].

5. Conclusions

Drug inhalation is a mainstay in the management of respiratory diseases. Its success not only depends on the pharmacology of the drugs being inhaled but also on the site and extent of deposition in the respiratory tract. Such deposition is tightly related to the physical properties of the inhaled aerosols (i.e., shape, size, density, electrostatic charge) and to the characteristics of the subject (i.e., lung geometry and size, breathing pattern, disease state). The upper airway is characterized by variable cross-sectional areas and sharp changes in flow direction that is conducive to deposition by inertial impaction. Most pharmaceutical aerosols target the intrathoracic lung and as such, deposition in the oral passages should be kept to a minimum to circumvent local adverse effects and to maximize lung dose.

Deposition of 1-30 µm particles was predicted in eleven models of oral airways of adults at two flow rates, one flow typical of resting subjects and one flow in the range recommended when using a DPI. This is the first report of in-silico predictions in such many geometries for both inhalation and exhalation. Averaged over all geometries, our data showed no significant difference in overall deposition in the oral cavity between the inspiratory and expiratory phase. In contrast, subregional patterns larger differed between the two phases, with areas of hot spots preferentially in the oropharynx during inspiration, and in the laryngeal region during expiration.

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**Data Availability Statement:** In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section “MDPI Research Data Policies” at https://www.mdpi.com/ethics. If the study did not report any data, you might add “Not applicable” here.

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**Appendix A – Individual geometries**

**Figure A1.** Upper airway geometry of all individuals.



**Table A1** lists the volume and surface area of each upper airway geometry shown in **Figure A1. T**he average cross-sectional area of the region between the mouth cavity and the larynx as defined in [9] is also listed in the table.

**Table A1**. Dimension of the oral airways.

|  |  |  |  |
| --- | --- | --- | --- |
| **Subject ID** | **Volume (cm³)** | **Surface Area (cm²)** | **Average cross-**  **sectional area (cm2)** |
| H1 | 73.70 | 188.18 |  |
| H2 | 65.32 | 178.55 |  |
| H3 | 63.88 | 179.94 |  |
| H4 | 96.34 | 210.22 |  |
| H5 | 67.92 | 193.32 |  |
| H6 | 72.51 | 200.18 |  |
| H7 | 61.28 | 192.96 |  |
| COPD1 | 52.86 | 149.79 |  |
| COPD2 | 126.83 | 254.02 |  |
| COPD3 | 85.53 | 243.09 |  |
| COPD4 | 87.40 | 220.84 |  |

Chart, histogram

Description automatically generated**Appendix B – Additional *in-silico* data (still need to decide what to include here)**

**Figure B1**. Subregional deposition as a function of particle size for both flow rates (dashed lines: 18 L/min, solid line: 45 L/min). (A) Mouth cavity. (B) Oropharyngeal region. (C) Laryngeal region.

A picture containing text, different

Description automatically generated**Figure B2**. Special regional distribution of deposited 5 µm particles inhaled at 45 L/min (A) Isometric view (B) Back view(C) Front view (D) Side view. Upper panel: subject H5; Lower panel: subject H6.

Figure B3: Special regional distribution of deposited 10 µm particles inhaled at 45 L/min (A) Isometric view (B) Back view(C) Front view (D) Side view. Upper panel: subject H5; Lower panel: subject H6.

References

1. Finlay, W. H. *The Mechanics of Inhaled Pharmaceutical Aerosols: An Introduction*. Amsterdam, NL: Academic Press, 2001.

2. Feng, Yu, Jianan Zhao, Clement Kleinstreuer, Qingsheng Wang, Jun Wang, Dee H. Wu, and Jiang Lin. "An in Silico Inter-Subject Variability Study of Extra-Thoracic Morphology Effects on Inhaled Particle Transport and Deposition." *Journal of Aerosol Science* 123 (2018): 185-207.

3. Borgstrom, L., B. Olsson, and L. Thorsson. "Degree of Throat Deposition Can Explain Variability in Lung Deposition of Inhaled Drugs." *J Aerosol Med* 19 (2006): 473-83.

4. Byron, P. R., M. Hindle, C. F. Lange, P. W. Longest, D. McRobbie, M. J. Oldham, B. Olsson, C.G. Thiel, H. Wachtel, and W. H. Finlay. "In Vivo-in Vitro Correlations: Predicting Pulmonary Drug Deposition from Pharmaceutical Aerosols." *J Aerosol Med Pulm Drug Deliv* 23 (2010): S59-S69.

5. Vinchurkar, S., L. De Backer, W. Vos, C. Van Holsbeke, J. De Backer, and W. De Backer. "A Case Series on Lung Deposition Analysis of Inhaled Medication Using Functional Imaging Based Computational Fluid Dynamics in Asthmatic Patients: Effect of Upper Airway Morphology and Comparison with in-Vivo Data." *Inhalation Toxicology* 24 (2012): 81-88.

6. Longest, P. W., and L.T. Holbrook. "In Silico Models of Aerosol Delivery to the Respiratory Tract—Development and Applications." *Adv. Drug Deliv. Reviews* 64 (2012): 296-311.

7. Cheng, Y. S. "Aerosol Deposition in the Extrathoracic Region." *Aerosol Sci Technol* 37, no. 8 (2003): 659-71.

8. Grgic, B., W. H. Finlay, P. K. P. Burnell, and A. F. Heenan. "In Vitro Intersubject and Intrasubject Deposition Measurements in Realistic Mouth-Throat Geometries." *J Aerosol Sci* 35 (2004): 1025-40.

9. Golshahi, L., M. L. Noga, R. Vehring, and W. H. Finlay. "An in Vitro Study on the Deposition of Micrometer-Sized Particles in the Extrathoracic Airways of Adults During Tidal Oral Breathing." *Annals of Biomedical Engineering* 41, no. 5 (2013): 979-89.

10. Cheng, Yung-Sung, Yue Zhou, and Bean T. Chen. "Particle Deposition in a Cast of Human Oral Airways." *Aerosol Science and Technology* 31, no. 4 (1999): 286-300.

11. Cheng, K.-H., Y.-S. Cheng, H.-C. Yeh, and D. L. Swift. "Measurements of Airway Dimensions and Calculation of Mass Transfer Characteristics of the Human Oral Passage." *Journal of Biomechanical Engineering* 119, no. 4 (1997): 476-82.

12. Gallivan, Gregory J, K Holly Gallivan, and Helen K Gallivan. "Inhaled Corticosteroids: Hazardous Effects on Voice—an Update." *Journal of Voice* 21, no. 1 (2007): 101-11.

13. DelGaudio, John M. "Steroid Inhaler Laryngitis: Dysphonia Caused by Inhaled Fluticasone Therapy." *Archives of Otolaryngology–Head & Neck Surgery* 128, no. 6 (2002): 677-81.

14. Xi, Jinxiang, Tiancheng Yang, Khaled Talaat, Tianshu Wen, Yu Zhang, Scott Klozik, and Shannon Peters. "Visualization of Local Deposition of Nebulized Aerosols in a Human Upper Respiratory Tract Model." *Journal of Visualization* 21, no. 2 (2018): 225-37.

15. Feng, Yu, Jianan Zhao, Hamideh Hayati, Ted Sperry, and Hang Yi. "Tutorial: Understanding the Transport, Deposition, and Translocation of Particles in Human Respiratory Systems Using Computational Fluid-Particle Dynamics and Physiologically Based Toxicokinetic Models." *Journal of Aerosol Science* 151 (2021): 105672.

16. Wedel, Jana, Paul Steinmann, Mitja Štrakl, Matjaž Hriberšek, and Jure Ravnik. "Can Cfd Establish a Connection to a Milder Covid-19 Disease in Younger People? Aerosol Deposition in Lungs of Different Age Groups Based on Lagrangian Particle Tracking in Turbulent Flow." *Computational Mechanics* 67, no. 5 (2021): 1497-513.

17. Tian, Geng, P Worth Longest, Guoguang Su, Ross L Walenga, and Michael Hindle. "Development of a Stochastic Individual Path (Sip) Model for Predicting the Tracheobronchial Deposition of Pharmaceutical Aerosols: Effects of Transient Inhalation and Sampling the Airways." *Journal of Aerosol Science* 42, no. 11 (2011): 781-99.

18. Zhang, Z, C Kleinstreuer, and CS Kim. "Micro-Particle Transport and Deposition in a Human Oral Airway Model." *Journal of Aerosol Science* 33, no. 12 (2002): 1635-52.

19. Schroeter, Jeffry D, Guilherme JM Garcia, and Julia S Kimbell. "Effects of Surface Smoothness on Inertial Particle Deposition in Human Nasal Models." *Journal of Aerosol Science* 42, no. 1 (2011): 52-63.

20. Tracy, Lauren F, Saikat Basu, Parth V Shah, Dennis O Frank‐Ito, Snigdha Das, Adam M Zanation, and Julia S Kimbell. "Impact of Endoscopic Craniofacial Resection on Simulated Nasal Airflow and Heat Transport." Paper presented at the International forum of allergy & rhinology 2019.

21. Borojeni, Azadeh AT, Guilherme JM Garcia, Masoud Gh Moghaddam, Dennis O Frank-Ito, Julia S Kimbell, Purushottam W Laud, Lisa J Koenig, and John S Rhee. "Normative Ranges of Nasal Airflow Variables in Healthy Adults." *International journal of computer assisted radiology and surgery* 15, no. 1 (2020): 87-98.

22. Frank-Ito, D. O., M. Wofford, J. D. Schroeter, and J. S. Kimbell. "Influence of Mesh Density on Airflow and Particle Deposition in Sinonasal Airway Modeling." *J Aerosol Med Pulm Drug Deliv* 29, no. 1 (2016): 46-56.

23. Li, C., J. Jiang, H. Dong, and K. Zhao. "Computational Modeling and Validation of Human Nasal Airflow under Various Breathing Conditions." *J Biomech* 64 (2017): 59-68.

24. Kelly, J. T., A. K. Prasad, and A. S. Wexler. "Detailed Flow Patterns in the Nasal Cavity." *Journal of Applied Physiology* 89, no. 1 (2000): 323-37.

25. Frank-Ito, Dennis Onyeka, and Seth Morris Cohen. "Orally Inhaled Drug Particle Transport in Computerized Models of Laryngotracheal Stenosis." *Otolaryngology–Head and Neck Surgery* 164, no. 4 (2021): 829-40.

26. Gosman, Raluca E, Ryan M Sicard, Seth M Cohen, and Dennis O Frank‐Ito. "Comparison of Inhaled Drug Delivery in Patients with One‐and Two‐Level Laryngotracheal Stenosis." *The laryngoscope* (2022).

27. Darquenne, C., W. J. Lamm, J. M. Fine, R.A. Corley, and R. W. Glenny. "Total and Regional Deposition of Inhaled Aerosols in Supine Healthy Subjects and Subjects with Mild-to-Moderate Copd." *Journal of Aerosol Science* 99 (2016): 27-39.

28. Asgharian, B., O. Price, A. A. T. Borojeni, A. P. Kuprat, S. Colby, R. K. Singh, W. Gu, R. A. Corley, and C. Darquenne. "Influence of Alveolar Mixing and Multiple Breaths of Aerosol Intake on Particle Deposition in the Human Lungs." *Journal of Aerosol Science* 166 (2022): 106050.

29. Stahlhofen, W., G. Rudolf, and A. C. James. "Intercomparison of Experimental Regional Aerosol Deposition Data." *Journal of Aerosol Medicine* 2 no. 3 (1989): 285-308.

30. Xi, J., and P. W. Longest. "Transport and Deposition of Micro-Aerosols in Realistic and Simplified Models of the Oral Airway." *Ann Biomed Eng* 35, no. 4 (2007): 560-81.

31. Chan, T.L., and M. Lippmann. "Experimental Measurement and Empirical Modeling of the Regional Deposition of Inhaled Particles in Humans." *Am. Ind. Hyg. Assoc. J.* 41 (1980): 399-409.

32. Lippmann, M. "Regional Deposition of Particles in the Human Respiratory Tract." In *Handbook of Physiology*, edited by D.H.K. Lee, H.L. Falk, S.D. Murphy and S. R. Geiger, 213-32. Bethesda, Maryland: American Physiological Society, 1976.

33. Foord, N., A. Black, and M. Walsh. "Regional Deposition of 2.5-7.5 Μm Diameter Inhaled Particles in Healthy Male Non-Smokers." *Journal of Aerosol Science* 9 (1978): 343-57.

34. Emmett, PC, RJ Aitken, and WJ Hannan. "Measurements of the Total and Regional Deposition of Inhaled Particles in the Human Respiratory Tract." *Journal of Aerosol Science* 13, no. 6 (1982): 549-60.

35. Stahlhofen, WJJG, J Gebhart, J Heyder, and G Scheuch. "New Regional Deposition Data of the Human Respiratory Tract." *Journal of Aerosol Science* 14, no. 3 (1983): 186-88.

36. Stahlhofen, W., J. Gebhart, and J. Heyder. "Experimental Determination of the Regional Deposition of Aerosol Particles in the Human Respiratory Tract." *Am Ind Hyg Assoc J* 41 (1980): 385-98.

37. Stahlhofen, W, J Gebhart, and J Heyder. "Biological Variability of Regional Deposition of Aerosol Particles in the Human Respiratory Tract." *American Industrial Hygiene Association Journal* 42, no. 5 (1981): 348-52.

38. Verbanck, S., H. S. Kalsi, M. F. Biddiscombe, V. Agnihotri, B. Belkassem, C. Lacor, and O. S. Usmani. "Inspiratory and Expiratory Aerosol Deposition in the Upper Airway." *Inhal Toxicol* 23, no. 2 (2011): 104-11.

39. Laube, B. L., H.M. Janssens, F.H.C. de Jongh, S.G. Devadason, R. Dhand, P. Diot, M.L. Everard, I. Horvath, P. Navalesi, T. Voshaar, and H. Chrystyn. "What the Pulmonary Specialist Should Know About the New Inhalation Therapies." *Eur Respir J* 37, no. 1308-1331 (2011).

40. James, A. C., W. Stahlhofen, G. Rudolf, R. Köbrich, J. K. Briant, M. J. Egan, W. Nixon, and A. Birchall. "Annexe D. Deposition of Inhaled Particles." *Annals of the ICRP* 24, no. 1-3 (1994): 231-99.

41. Li, J. T., and C. E. Reed. "Proper Use of Aerosol Corticosteroids to Control Asthma." *Mayo Clin Proc* 64, no. 2 (1989): 205-10.

42. Williamson, I. J., S. P. Matusiewicz, P. H. Brown, A. P. Greening, and G. K. Crompton. "Frequency of Voice Problems and Cough in Patients Using Pressurized Aerosol Inhaled Steroid Preparations." *Eur Respir J* 8, no. 4 (1995): 590-2.

43. Schwab, R. J., W. B. Gefter, A. I. Pack, and E. A. Hoffman. "Dynamic Imaging of the Upper Airway During Respiration in Normal Subjects." *J.Appl.Physiol* 74 (1993): 1504-14.

44. Darquenne, C., A. R. Elliott, B. Sibille, E.T. Smales, P.D. DeYoung, R. J. Theilmann, and A. Malhotra. "Upper Airway Dynamic Imaging During Tidal Breathing in Awake and Asleep Subjects with Obstructive Sleep Apnea and Healthy Controls." *Physiological Reports* 6 (2018): e13711 (pp1-9).

45. Yoshida, T., R. Kondo, and T. Horiguchi. "A Comparison of Posterior Pharyngeal Wall Areas between Different Tongue Positions During Inhalation." *J Allergy Clin Immunol Pract* 7, no. 2 (2019): 743-45.e1.

46. Horiguchi, T., and R. Kondo. "Determination of the Preferred Tongue Position for Optimal Inhaler Use." *J Allergy Clin Immunol Pract* 6, no. 3 (2018): 1039-41.e3.

47. Heenan, A. F., W. H. Finlay, B. Grgic, A. Pollard, and P. K. P. Burnell. "An Investigation of the Relationship between the Flow Field and Regional Deposition in Realistic Extra-Thoracic Airways." *Journal of Aerosol Science* 35, no. 8 (2004): 1013-23.