Incoming Flow Turbulence Reduction Increases the Efficiency of Aerosolized Medication Delivery to 3D Oral Mucosal Tissue Models.

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Research

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Abstract

Background: Inhalable medication devices on the market deliver aerosolized drugs in a turbulent flow, which creates significant obstacles for reaching the lower lungs. The complexity of the interaction of external turbulent flow from an inhalation device with complex anatomy of the upper airways, including the oropharyngeal cavity, makes the fidelity of aerosolized medication delivery to the lungs extremely low. Unpredictable outcomes, waste, and side effects result from unintended upper airway drug deposition.

Methods: Here we compared the efficiency of aerosolized medication (fluticasone) delivery via a novel Flow Modification Device (ModiFlow) to that of a Standard Spacer (SS) device. The ability of ModiFlow to minimize the turbulence in the flow was assessed preliminarily by measuring the length of the Laminar Outflow using video recording. Oral mucosal 3D tissue culture (SkinAxis) was used as a target for delivery, and was placed either “well within” the range of the length of the oro-pharyngeal cavity (5cm) or “well outside” of it (20cm) from exit points of each device. The efficiency of fluticasone delivery to the surface of tissue cultures was quantified by mass spectrometry.

Results: The results of the study demonstrated a statistically significant advantage of ModiFlow over a Standard Spacer in delivering aerosolized fluticasone to target tissue at both distances. The difference in the efficiency of delivery between the two spacers was more pronounced at a longer distance.

Conclusions: Lamination of the outflow using internal septi helps improve the delivery of aerosolized medication to target tissue despite an increased inner surface area of the spacer device, which also indicates lesser deposition of the medication on the walls of the test tube. This suggests that the use of ModiFlow will potentially result in the more efficient delivery of aerosolized medications to the lungs with lesser deposition in the oral cavity and fewer side effects.

Background

In recent years the targeted aerosol delivery to the respiratory tract has rapidly gained interest as the preferred route for the treatment of lung diseases locally, as well as accessing the systemic circulation. Many therapeutic aerosols containing large molecules - proteins, hormones, nucleic acids, chemotherapeutic drugs [1, 2], have shown a promise as agents for gene therapy, antiviral therapy for influenza and measles, insulin and vaccine delivery [3, 4].

Our increasing understanding of medication receptor distribution throughout the lungs further emphasizes the need for delivering different aerosolized medications to very specific areas of the respiratory tract [5]. In recent years more than 100 different medications have been brought to the market in the aerosolized form [6]. However, the efficiency of aerosolized medication delivery to the lungs remains extremely low due to significant variability in parameters within the upper respiratory tract and the design of the delivery devices. Consequently, up to 77% of the medication can deposit in the upper airways [7], most notably in the oral and pharyngeal areas, thus not only reducing efficiency and lowering

the predictability of the outcomes but also causing side effects. With the entrance of newer inhalable medications to the market, new delivery standards are being explored [8, 9].

The emergence of turbulent flow in different segments of the respiratory tract can have a significant impact on the deposition and absorption of the aerosols. Although largely overlooked, for many aerosolized medications this may be a factor of critical importance. The presence of turbulence can increase the localized shear wall stress [10], create significant resistance to the flow, and reduce farther propagation of the aerosolized substances. To our knowledge, no significance has ever been given to turbulence emerging from the delivery device itself as a major obstacle to the effective delivery of aerosols to the lungs. Lamination of the device outflow with a reduction of turbulence to optimize its interaction with oropharyngeal inspiratory airflow may have a positive impact on the efficiency of aerosol delivery to the lungs. Even with the use of spacers, despite the variations of “external” parameters to improve their performance, except for volume, the overall efficiency of aerosol delivery to the lower lungs remains poor[11–13]. The inner space of a spacer device, where the flow of aerosolized medication occurs, offers significant opportunity for manipulating the flow [14]. Most, if not all of the existing spacers on the market lack any internal structures, and the exiting flow of aerosol is highly turbulent.

A device that can reduce the turbulence in the aerosolized medication outflow may prove to be more efficient in delivering the medication to the lower lungs and reducing its deposition in the upper airways.

ModiFlow is a novel spacer-like delivery device specifically designed to create a Laminar Outflow of aerosolized medication (Flow Modification Device, G. Greg Haroutunian, MD, US Patent # 8,371,291 B2). Modeling of the airflow and particle deposition rates in the airways is often done with the use of Computational Flow Dynamics (CFD) methods [15–17]. However, the extreme complexity of parameters and factors to be accounted for naturally limit the capabilities of these methods. More direct methods of assessing the medication deposition and absorption rates could be obtained on actual 3D tissue samples. In recent years progress has been made in constructing 3D tissue models and using them for various research and clinical applications [18–21]. This allowed for departure from the use of animal tissue models, and for obtaining data in realistic physiologic conditions[21–27].

Materials And Methods

Aerosol drug delivery devices

In this study, we compared the performance of the ModiFlow (MF) to an idealized Standard Spacer (SS). For standardization purposes, a hollow cylindrical tube of identical length and inner diameter represented both devices, with the only difference being that MF had specifically designed inner septal structures, which SS didn’t.

Since most, if not all spacer devices on the market have a cylindrical shape, and no internal structures, our Standard Spacer model was considered to be a fair representation of spacers on the market. In addition, several studies have demonstrated that with the exception of volume, all other structural

modifications to the spacers, such as valves and masks, have not made a significant difference in their performance. Regardless, our goal was to minimize any structural variability between the tubes for the purpose of controlling all parameters, and leaving only one main difference – the presence of inner septal structures in MF, and the absence thereof in SS.

In accordance with the above, for this study the parameters for ModiFlow were selected as follows: Total length – 100mm, Inner Diameter – 38mm, Septi – 62mm, 34mm, 24mm. The calculated Inner Surface Area (ISA) was 164.9 sq. cm, of which 45.6 sq. cm is accounted for Septi only, and 119.3 sq. cm for inner walls. As a SS, a hollow cylindrical tube of the same Length (100mm) and Inner Diameter (38mm) as in MF was used, with the calculated ISA of 119.3 sq. cm.

To compare the efficiencies of aerosol delivery for ModiFlow vs a Standard Spacer, 4 different Test Tubes (TT) have been devised, all of which had a similar structure as depicted in **Figure 1**. Two of them had a length of 15cm, and two – 30cm. All four TT's were of cylindrical shape, and of the same inner diameter (38 mm). In the TT of each length, either ModiFlow (**Figure 1-5**) or a Standard Spacer (**Figure1-1**) were inserted flush with the proximal end of the TT. The proximal end of TT's was furnished with an adapter (4) for Medication Pump (5), and the distal end – with a receptacle (1) for tissue culture. This set the distances from the exit of the spacer (MF or SS) to the distal end of TT (where the tissue culture was placed) to be either 15-10=5 (cm) or 30-10=20 (cm), thus creating four different designs of TT: MF-5, MF-20, SS-5, SS-20. This distances from the spacer exit to the tissue culture were selected to be either "within" (5 cm) or "outside" (20 cm) of the average range of adult oro-pharyngeal cavity. This would allow for four pairs of comparisons to be made: MF-5 to SS-5, MF-5 to MF-20, MF-20 to SS-20, and SS-5 to SS-20. The total ISA for each Test Tube was respectively MF-5 – 224.6 sq. cm, MF-20 – 403.5 sq. cm, SS-5 – 179 sq. cm, SS-20 – 358 sq. cm, excluding the surface area of tissue cultures and receptacles.

Aerosolized medication

Fluticasone Propionate Metered Dose Inhaler (220 µg per spray) was used in all test tubes.

3D Oral Epidermal tissues

To better approximate physiological conditions of drug delivery, we measured aerosolized drug deposition on a SkinAxis model of the oral mucosal tissues. Normal human Gingival keratinocytes (SkinAxis) were cultured on specially prepared cell culture inserts using serum-free medium and differentiated in vitro using proprietary SkinAxis' cell culture technology to form multilayered, highly differentiated models of the human gingival phenotypes (**Figure 3**, and www.skinaxis.com). SkinAxis oral epidermal tissue models are highly reproducible and exhibit in vivo-like morphological and growth characteristics. The differentiated tissue was inserted at the end of the spacer, as described above and to quantify drug deposition tissues were processed for Mass Spectrometry.

Fluticasone extraction from oral tissues and quantification.

Each tissue sample was processed by: adding 50 ml 0.1% formic acid and 200ml of methanol to a culture plate, scrapping with pipette tips, and transferring to an Eppendorf tube. The plate was washed sequentially with 200ml 0.2% formic acid and 100ml methanol and the washes combined with the initial extract. Extracts were sonicated for 1 min and centrifuged for 5 min at 25000 x g. Supernatants were diluted 10-fold using 50% methanol/0.1% formic acid before analysis by LC-MS.

HPLC–MS experiments were performed using a ThermoFisher Velos LTQ Orbitrap Pro mass spectrometer interfaced with a Dionex U3000 chromatography system. Samples (5 µL) were injected in microliter pick up mode and separated on a reverse-phase column (Discovery BIO Wide Pore C18, 5cm x 2.1mm, Supelco Analytical). Chromatography was conducted at a flow rate of 200µl/min using a gradient formed with an aqueous solution of 0.2 % acetic acid (solvent A) and methanol (solvent B) as follows: 60% B (1 min), 60-90% B (linear increase in 3 min), 90% B for 1 min, 90- 60% B (linear decrease in 0.1 min), and equilibration at 60% B (3 min). The column temperature was maintained at 45°C. MS acquisition parameters were as follows: the electrospray ion source was operated in positive ion mode (ESI+). The positively charged fluticasone (m/z= 501.3) was isolated in the ion trap with an isolation window of 3 m/z and fragmented with CID with a relative collision energy of 25% and activation time of 10 milliseconds. Fragments were detected using the ion trap and the 303.15 m/z fragment used for quantification.

A standard curve consisting of dilutions of fluticasone in methanol (0.01 ng/ml to 100mg/ml) was analyzed in parallel with samples. Peak areas of the 501-301 transitions were measured using XCalibur software. Concentrations of fluticasone in samples were determined with respect to standard curves by non-linear regression using a four-parameter sigmoidal fit, weighted by 1/y^2.

Statistical analysis

For each of the two spacers, independent *t*-tests were used to test the effects of varying drug deposition in different experimental settings. *P*-value < 0.05 was considered statistically significant.

Results

Comparing the efficacy of drug delivery of Standard Spacer to ModiFlow.

The geometry of ModiFlow inner space is structured using uniquely designed longitudinal septi of different lengths that produce a laminar (non-turbulent) flow, which persists after exiting the device (82-179 mm in one particular model).

Subdivision of the main flow into two sub-flows iterated multiple times (a fractal tree), helps to disrupt the cycle of growth of the lateral force, preventing the emergence of a high Reynolds number turbulence. This

tree-like branching pattern generated by iteratively applying a set of simple rules is pervasive in biological networks and is also utilized to model lung function and the bronchial tree [28, 29]. In ModiFlow the septi serve to prevent the emergence of turbulence by periodically sub-dividing the flow, and with it, dividing the lateral force that grows in an iterative side-to-side movement, and would eventually break the flow into a turbulent one. At the same time, they reduce the mixing of sub-flows, with their larger surface area they filter out larger size droplets that tend to deposit on the upper portions of the respiratory tract mucosa, and also remove the tangential (lateral, non-parallel) parts of the flow as well. As a result, the aerosol flow emerges from the device more laminar, coherent, unidirectional, and with more uniform particle size. The lengths of the septi and the total length of the ModiFlow tube are selected to be proportional to sequential Fibonacci numbers (these are numbers in a sequence in which each number is a sum of two preceding ones), assuring that their ratios are close to “golden ratio” = 1.618 (it is known that if a Fibonacci number is divided by its immediate predecessor, the resulting quotient approximates 1.618). There is some evidence that this particular ratio is effective in keeping the flow laminar.

This improved laminar flow can have potentially significant implications: 1) There is a higher likelihood of such a laminated flow to overcome the oro-pharyngeal barrier without depositing most of the particles there, a larger portion of the flow will reach trachea and main bronchi; 2) The higher degree of lamination of the flow will skew the distribution of aerosol particles pattern towards deposition in lower parts of the respiratory tract, including finer bronchioli and alveoli; 3) The manipulation of the geometry of ModiFlow could allow a more targeted deposition of particles in specific areas of respiratory tract depending on the need and type of medication being delivered and the distribution map of its receptors in the airways; 4) Reduction of side effects due to reduction of deposition in the mouth, throat, trachea and upper bronchi; 5) Improved/more efficient delivery may overall reduce the cost of expensive medications.

To assess the ability of MF and SS to laminate a flow, multiple measurements of the Laminar Outflow (the portion of the exit flow that remains continuously laminar) were taken using video-recording of a smoke flow via both spacers. For ModiFlow the measurements indicated that the flow remained completely laminar in the range from 82 to 129 mm, transitioned to non-laminar between 82 and 203 mm, and was completely non-laminar above 179-203 mm from the exit point (**Figure 2**). This was significantly more extended laminar flow than for Standard Spacer could be achieved, which remained in the ranges of less than 30 mm. This data was only obtained and used for the purpose to demonstrate the significantly better ability of the ModiFlow to laminate the flow. No attempts were ever made to correlate these measurements with the outcomes of the study.

The tissue samples were inserted at the distal end of each Test Tube (TT) through an adaptor (see **Figure 1**, membrane holder), perpendicular to the axis of the TT (axis of flow), opposite to the end where the medication dispenser was inserted. ModiFlow or Standard Spacer were incorporated flush with the proximal end of the TT, and oriented in such a way that the flow is directed towards the tissue sample (**Figure 1**). The positioning of the tissue sample perpendicular to the axis of flow at different distances allows for the assessment of aerosol flow (delivery) through a particular cross-section of the TT. Placing the tissue samples on the lateral walls of the TT would make it very difficult to discern any correlation

between the aerosol flow and its deposition on the tissue sample, having to take into account too many variables.

Experiments were conducted to maximally mimic the real-life application of aerosol oral delivery of Fluticasone. After shaking for 5 seconds and spraying away once to assure proper functioning, the Fluticasone Metered Dose Inhaler was inserted into the medication pump adapter of the Test Tube containing the target tissue and was activated twice with a 3-minute interval by pushing the top of the medication canister all the way down. This delivered a total dose of 440 µg (220 µg per spray) in accordance with standard dosing recommendations. Because of the hermetic closure of Test Tubes, and of the same standard amount of Fluticasone delivered into each TT, it was possible to not only measure the amount of Fluticasone delivered along the axis of flow to the tissue samples at different distances but to also indirectly assess the amount of Fluticasone deposited on the lateral walls of each TT. This could be achieved by subtracting the amount of Fluticasone deposited on each tissue sample from the total dose of 440 µg sprayed in each TT.

The aerosol droplet sizes and the flow velocities were controlled by using the same Fluticasone MDI in all Test Tubes.

To minimize possible electro-static influences, all test tubes and spacers were pre-washed with standard dishwasher detergent.

The results presented in **Figure 4** indicates that ModiFlow significantly increased drug delivery to the tissue as compared to the Standard Spacer. ModiFlow delivered on average 20.19 µg and 8.9 µg of drug/tissue at 5 cm and 20 cm distance, while Standard Spacer delivered 7.8 µg and 3.05 µg/tissue respectively (**Figure 4**), strongly suggesting a more efficient Fluticasone delivery to mucosal tissue by ModiFlow.

Discussion

The efficient delivery of aerosolized medications to the patient's airways continues to be a challenging task. Various devices such as pumps, spacers, and nebulizers are currently used for that purpose. In all of the devices on the market, the outflow of aerosolized medication is significantly turbulent. Turbulent flow is one of the main reasons for the deposition of up to 77% of the medication in the upper airways [7, 10]. The interaction of the outflow of a delivery device with the complex geometry of the oropharyngeal segment of the upper airways may further contribute to these inefficiencies. To our knowledge, no specific measures have been taken so far to modify the outflow of these devices with the intention to reduce the turbulence. Accurately assessing the efficiency of laminated aerosol delivery may yield important results.

It is important to note that all 4 Test Tubes with attached medication pumps constituted a closed system, and the amount of Fluticasone sprayed into each one of them was identical. Since the amount of Fluticasone that reached the cell cultures at the end of Test Tubes was significantly higher (2.5-3-fold) with ModiFlow than with Standard Spacer, therefore smaller amounts were deposited elsewhere in Test

Tubes with ModiFlow. Also, although both the ModiFlow and the Standard Spacer have the same length and diameter, the Inner Surface Area of the Standard Spacer is 119.3 sq. cm, while that of the ModiFlow is 38% larger – 164.9 sq. cm due to the presence of inner septi. This would cause a larger amount of Fluticasone deposition within ModiFlow as compared to the Standard Spacer.

As indicated above, the delivery of the aerosolized Fluticasone as measured by the tissue surface deposition rates was significantly more effective via ModiFlow than via Standard Spacer in both distance ranges – short and long ($p < 0.01$ and $p < 0.05$ respectively). Interestingly, the comparative efficiency of ModiFlow can be judged to be higher in the long-range than in the short-range if ratios of deposited Fluticasone are used: 8.89/3.05 = 2.91 (long-range) > 20.18/7.79 = 2.59 (short-range). This may be due to 2 different factors: 1. The difference in the lengths of Laminar Outflow between MF and SS is more pronounced in longer Test Tubes. 2. The share of ISA of Septi in Total Test Tube ISA is almost twice lower in longer Test Tubes with MF (45.6/403.5 = 0.113) than in shorter ones (45.6/224.6 = 0.203).

From here it may be deduced with some level of certainty that not only higher amounts of Fluticasone are being delivered to target tissues via ModiFlow compared to Standard Spacer, but also smaller amounts of it are being deposited on the walls of the Test Tube idespite of the higher ISA in ModiFlow.

Conclusion

Turbulence plays a significant role in reducing the efficiency of aerosolized medication delivery to the lungs. In the ModiFlow device the inner space is structured in a manner that helps laminate the outflow and reduce the emergence of turbulence in the oropharyngeal cavity. This study demonstrated that despite the larger inner surface area, ModiFlow increases the efficiency of aerosol delivery to farther distances, and reduce its deposition in anterior/upper airways. This becomes increasingly important with newer aerosolized medications entering the marketplace with high potential for side effects, high cost of medication waste, higher need for efficient, consistent and targeted delivery.

Abbreviations

ModiFlow (MF); Standard Spacer (SS); Three Dimensional (3D) oral mucosal tissue models; Test Tubes (TT).

Declarations

Acknowledgments

Not applicable

Availability of data and materials

The datasets and materials used in the current study are available from the corresponding author upon request.

Author's contributions

GGH and AM designed the study, analyze data and wrote the manuscript, HZ and AT performed experiments and analyze the data. All the authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing Interests

Greg Haroutunian is the inventor of ModiFlow (US Patent # 8,371,291 B2).

All authors declare that they have no competing interests.

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Figures

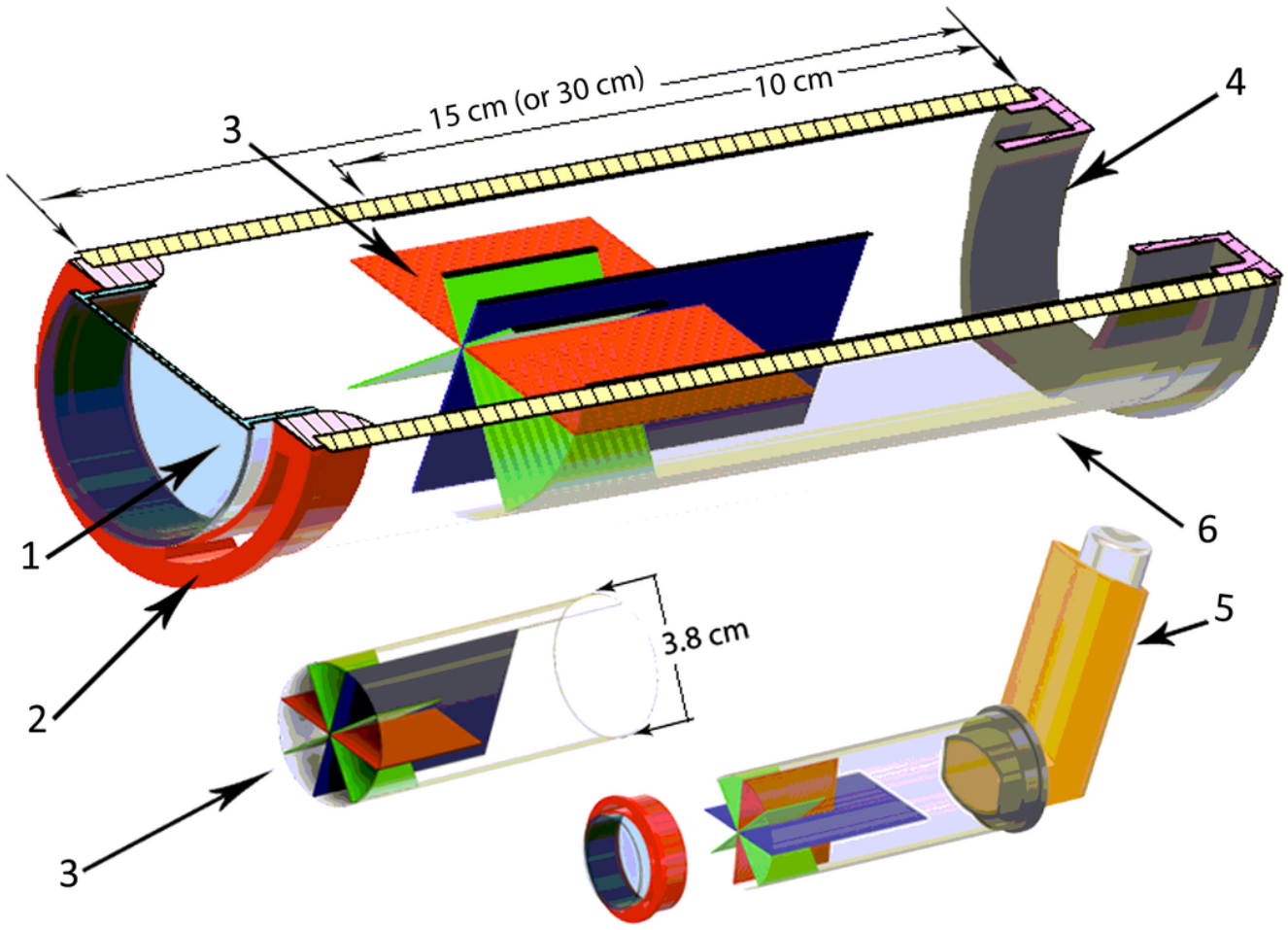
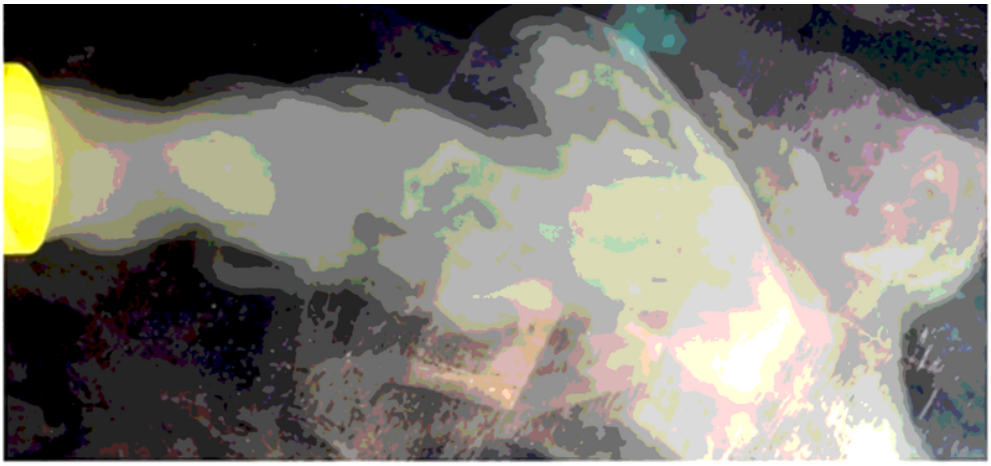


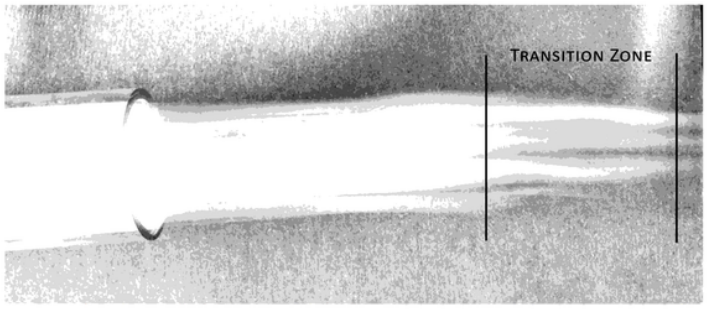
Figure 1

ModiFlow design with expanded view of the septated spacer (top). Total length:100mm. Septi: 62mm, 34mm, 24mm. The main components of the device are indicated: 1) Exit (cell target membrane); 2) Membrane holder; 3) Septi; 4) Medication pump adapter; 5) Medication pump; 6) Spacer tube.



Currently used Standard Spacer

Laminar Outflow (range: 82-129 mm)



Transition from laminar to turbulent flow

Figure 2

ModiFlow produces a longer/more constant laminar flow. Currently available spacers produce highly turbulent flows (Top). Laminar flow is generated from ModiFlow over a significant distance from exit (bottom).

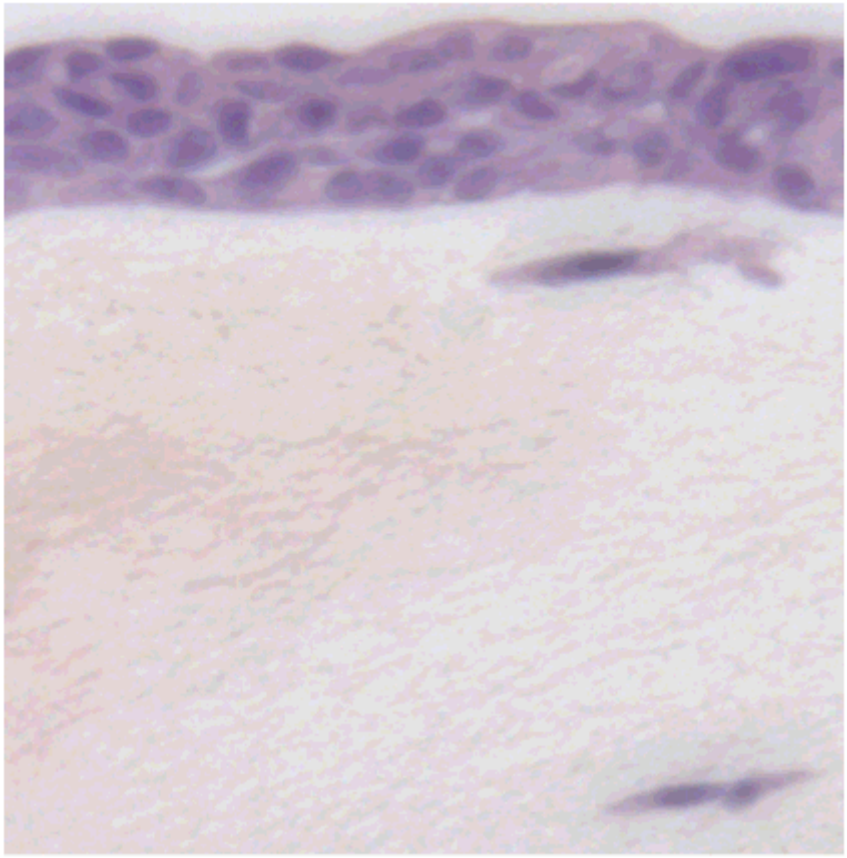


Figure 3

Reconstituted three-dimensional oral mucosa from primary cells reproduces the histological characteristics of the normal tissue. Stratified keratinocyte layers and collagenous lamina propria with fibroblasts are visible. Original magnification 200X.

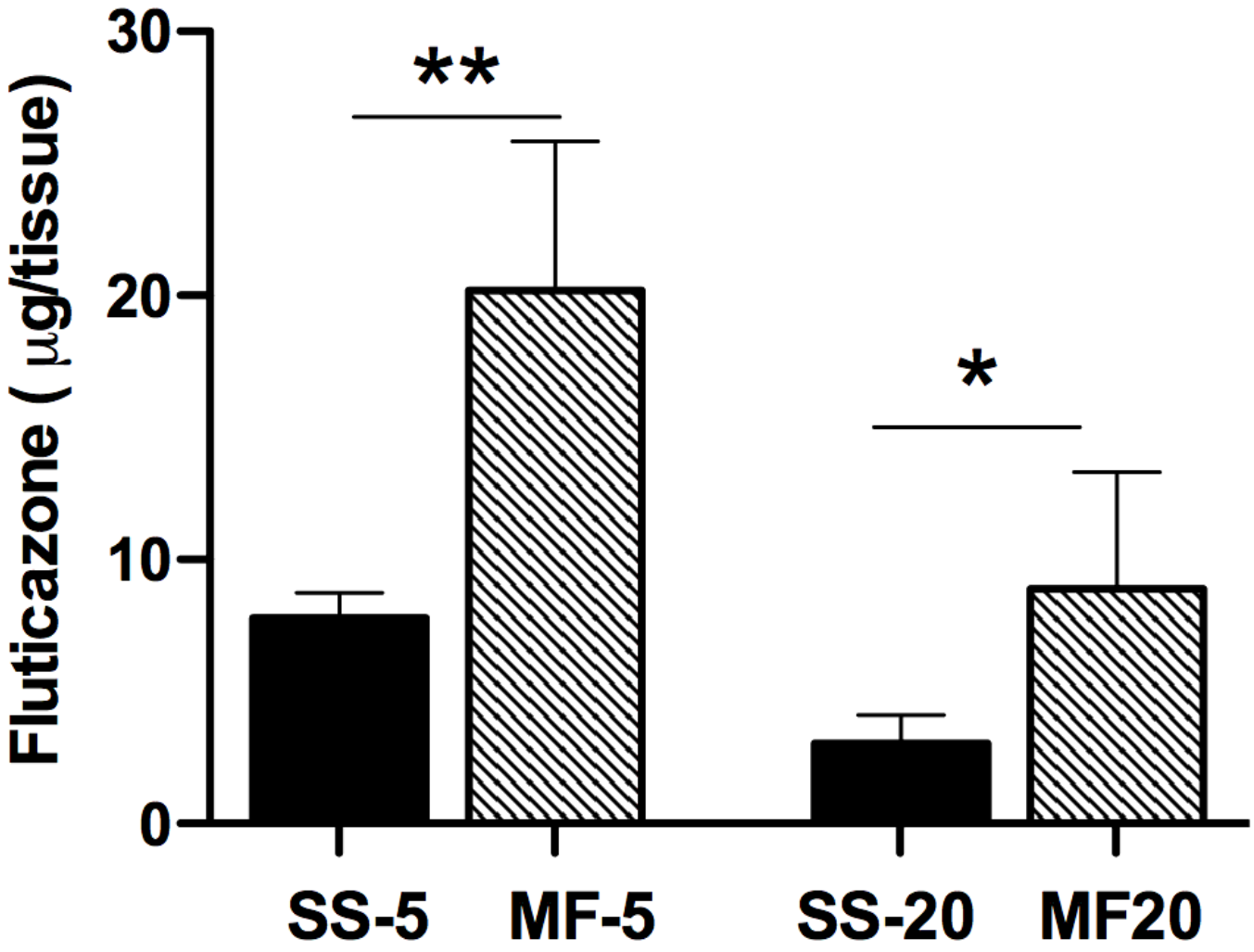


Figure 4

Different delivery efficiencies of Fluticasone to 3D oral mucosa tissue models. Absorption of aerosolized Fluticasone on live tissues upon delivery with Standard Spacer (SS) and ModiFlow (MF) at 5cm and 20cm distance from spraying device. *p<0.05; **p<0.01 by t test.