Use of reconstructed tissue and 3D printed CT-based nasal casts for the assessment of nasal delivery.

Amegashie, A. LaBarge, W. Collins, AV. Resek, A. Volmer, JB

ABSTRACT

Development of new drug products for delivery via the respiratory system for local or systemic effect can be difficult and risky due to complications in distribution, permeation and penetration, and clearance. Traditionally, these parameters have been de-risked during development through the use of physical models of the nasal airway to investigate formulation distribution and excised animal tissue to investigate permeation, penetration, and clearance. These models are known to have their specific limitations. Excised animal tissue is typically frozen prior to use and is nonviable. Physical airflow models are idealized, and have nonadhesive surfaces, which allow formulation to shift position after delivery due to gravity. Results from these studies can be inconclusive, or even misleading. In this poster, we describe the use of reconstructed nasal airway for the assessment of penetration & permeation, and the use of a novel CT scan-based 3D printed nasal cast for the assessment of distribution. Reconstructed Nasal Epithelium (RNE) is produced from primary nasal epithelial cells collected from normal healthy epithelium at the mid-turbinate level. These constructs can be fully differentiated into nasal epithelium with tight junctions, mucus production, metabolic activity, and ciliary action. Here, we show that while assessment on processed ovine tissue yields nonsignificant (and potentially misleading) results, reconstructed tissue can provide better insight. Nasal cast technology often ignores the importance of drug binding to cast material and is limited in what regions can be assessed and compared. 3D printed nasal casts generated from CT scans allow custom segmentation of the nasal cavity, and materials flexibility, to allow for optimization of drug binding and recovery during the assessment.

RECONSTRUCTED NASAL EPITHELIUM

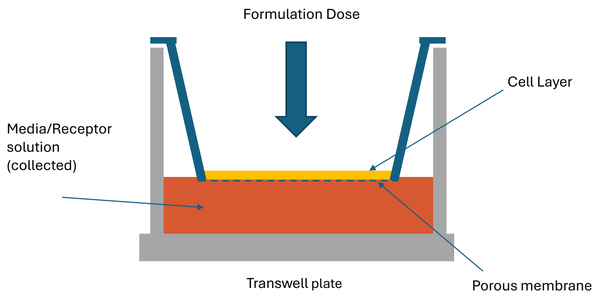


Figure 1, Reconstructed epithelium. Primary human nasal or bronchial epithelial cells are plated on a Transwell membrane and expanded to a confluent monolayer using a specific epithelial growth media. When the cells reach full confluency, the media is replaced on the basolateral side with a specific differentiation media. The apical side is left open to air. As the cells differentiate, they form a mucus layer, tight junctions, and motile, coordinated cilia. Differentiation is tracked by morphologic changes under light microscopy as an indication of cilia development and mucus layer, and by increase in trans epithelial electrical resistance (TEER) as an indication of tight junction formation & barrier function.

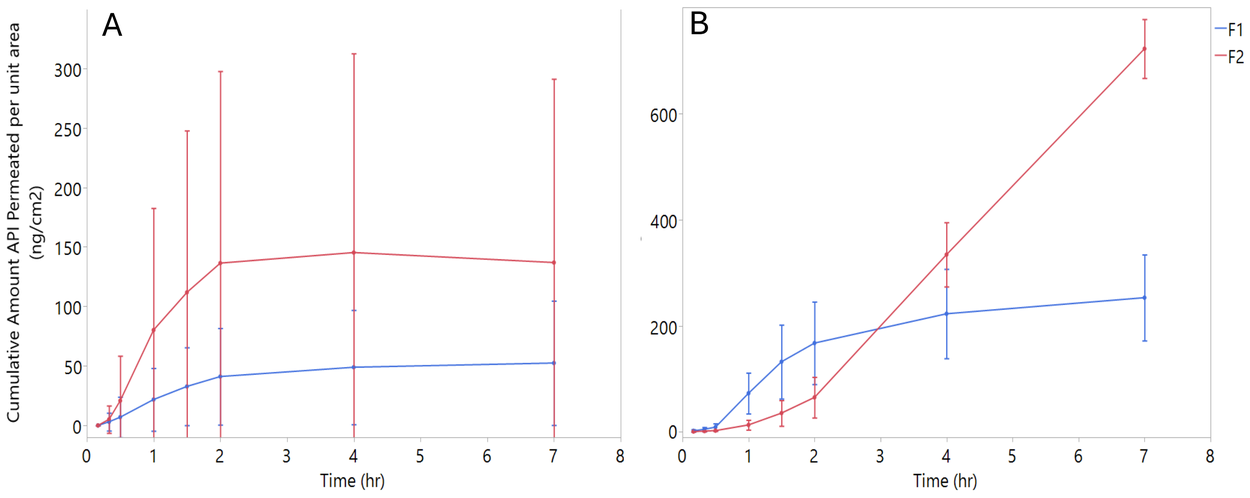


Figure 2. Reconstructed nasal epithelium compared to frozen ovine epithelium for mucosal permeation testing. Ovine mucosa was dissected from sheep cadaver head septa, cut to appropriate size, and mounted on a 2mL Franz diffusion cell with a surface area of 0.6 cm^2. Formulations 1 and 2 were applied, and samples were collected from the basolateral side at the indicated intervals (Fig2A). In parallel, reconstructed nasal epithelium (RNE) was prepared as described. Formulations were applied apically, and receptor solution was collected basolaterally at the indicated timepoints (Fig 2B). Although no statistical differences were noted in the ovine mucosa, many drug development programs would tend to select F2 as the higher trending formulation, despite the analysis. Testing with reconstructed epithelium yields a more complex and informative result. Although F2 does outperform F1 by the 4h timepoint, the early timepoints (<2h) indicate improved delivery from F1 during the most relevant time period for nasal delivery. Furthermore, results from RNE are statistically significant ($p<0.05$).

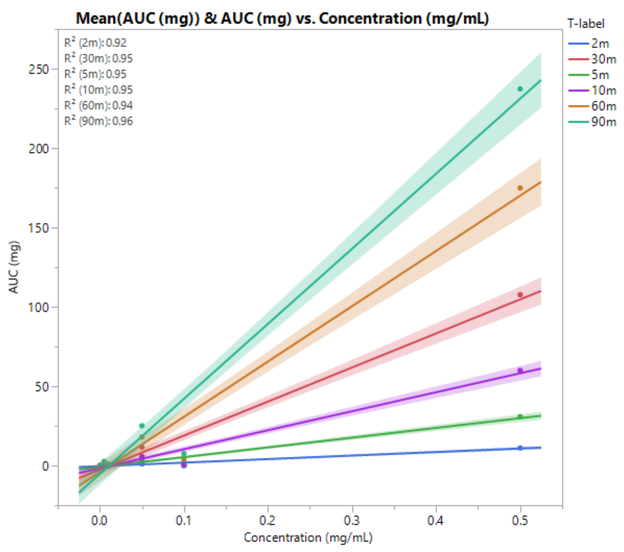
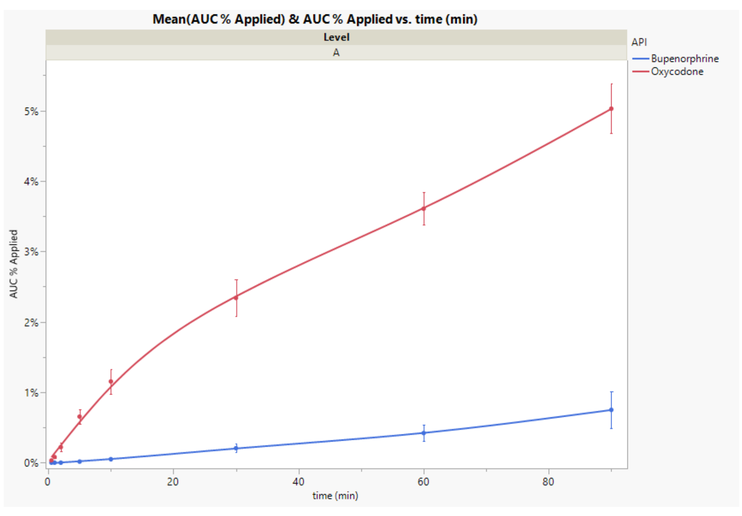


Figure 3. Formulations of oxycodone were prepared at varying concentrations ranging from 0.5 ug/mL to 0.5 mg/mL. Formulations were applied to RNE cultures, and receptor solution was sampled at timepoints from 2 – 90 minutes. Given the physiochemical properties of the API and formulation, total cumulative amount is expected to primarily driven by concentration across this range. Total cumulative amount was plotted against concentration for each time point, and linearity was calculated. All tested timepoints showed strong correlation for concentration to total cumulative amount.



Oxycodone[1], 0.1 mg/kg			Buprenorphine[2], 0.3mg	
Subject	Cmax (ng/mL)		Subject	Cmax (ng/mL)
1	17		1	0.67
2	6		2	0.64
3	8		3	0.67
4	12		4	2
5	14		5	4.78
6	6		6	2.54
7	7		7	1.92
8	15		8	0.76
9	20		9	1.93
10	25			
Mean	13		Mean	1.77
SD	6.1		SD	1.27

[1]Acta Anaesthesiol Scand. 1997 Feb;41(2):309-12

[2]J Pharm Pharmacol. 1989 Nov;41(11):803-5

Figure 4. Formulations of Buprenorphine and Oxycodone were prepared and apically delivered to RNE cultures. Samples were collected from the basolateral side at the indicated time points and analyzed for total cumulative amount. These results were compared to Cmax observations from similar formulations used in a clinical study of nasal delivery of oxycodone[1] and buprenorphine[2]. The CMax ratio of oxycodone : buprenorphine in the clinical study was 7.34. Total cumulative amount ratio of oxycodone : buprenorphine in the *in vitro* study was 7.14, suggesting a relative predictive value for the model.

CT BASED DISTRIBUTION MODEL (MEDCAST)

Figure 5, Medcast. Nasal cast models can be very useful for the testing of devices for delivery to specific parts of the nose. Existing models are based on idealized & simplified internal structure and are made using standardized materials and segmentaion. 3D printing technology allows for the construction of nasal casts with internal structures that closely mimic the internal structure of the nasal cavity, custom segmentation of the nasal cats to focus investigation to specific regions of interest, and the construction of the casts out of varying materials for API and formulation compatibility. The MedCast is based on a 3-dimensional model of human interna nasal structure obtained from CT scans. It can be custom segmented and printed from materials tested for compatibility with specific drug & excipient combinations. The device can be fitted to an artificial lung, allowing for simulated inhalation, and can be fitted to an NGI to assess distribution further down the respiratory tract.

Figure 6. CT scans were taken of normal, healthy volunteers. These were processed using 3D modelling software and divided using anatomical cues into nares, turbinates, pharynx, olfactory bulb, and various sinuses. A seal structure was added to each segment to ensure a fully airtight structure that would allow airflow as expected in a healthy nasal passage. Segments are represented in figure 6A allow for differentiation between pharynx, sinuses, turbinates, olfactory bulb, and nares. Figure 6B highlights detail of the internal turbinate region.

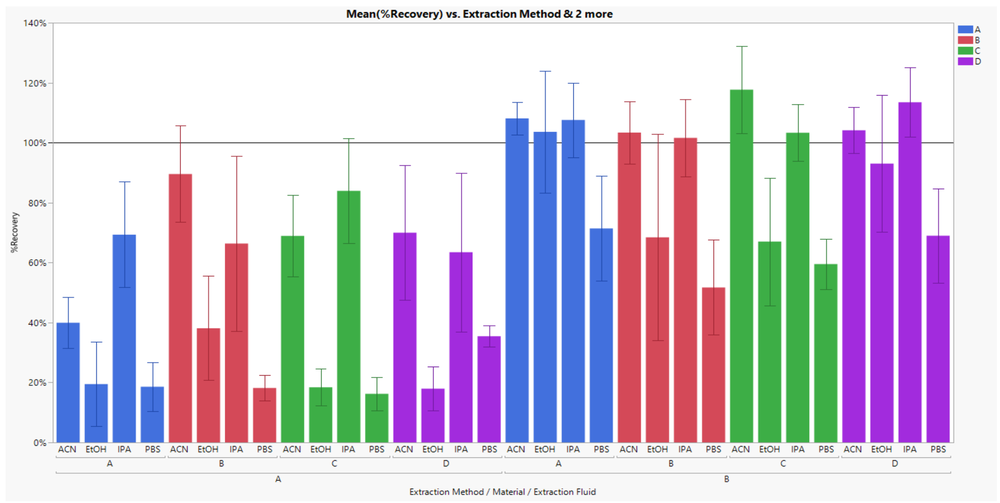
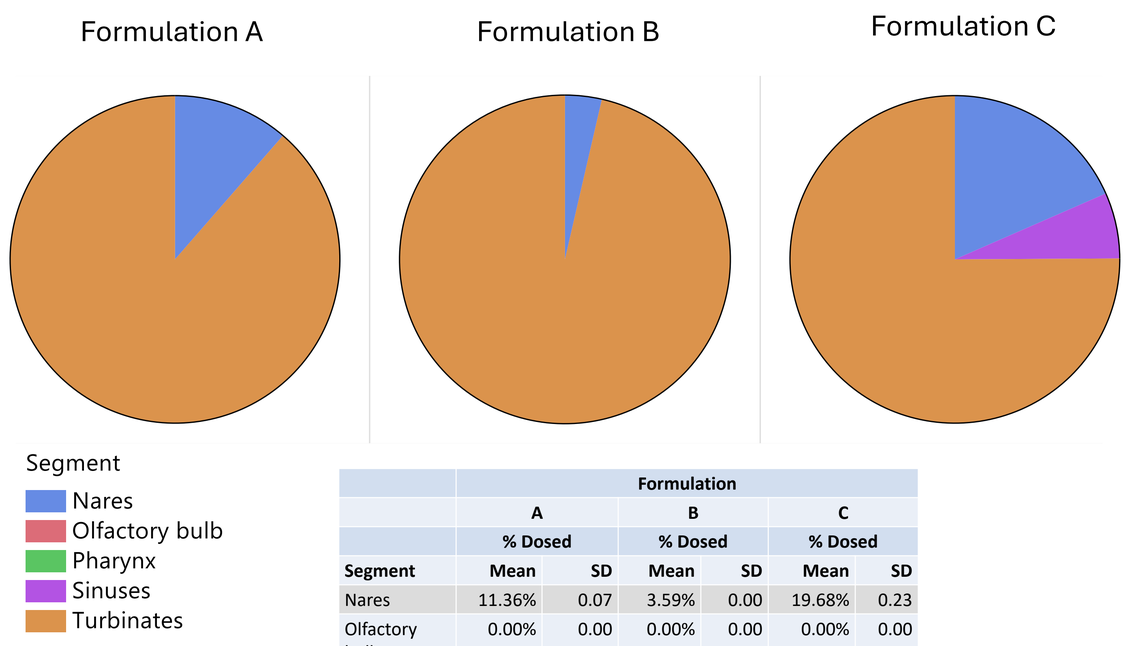


Figure 8. *In vivo*, administered formulation is trapped and then cleared by the mucus layer. In a nasal cast model, care must be taken to ensure formulation that comes in contact with the interior of the cast does not flow to other regions, and that drug, once adsorbed, can be extracted. This is largely dependent on the API being delivered, and on the formulation used. To this end, small samples of four candidate 3D printing materials (A,B,C, and D) were plasma treated using two methods (A and B). A known ammount of drug (dicflofenac) was deposited on the material, and was extracted using four methods (ACN, EtOH, IPA and PBS). Extracted drug was analyzed by LC-MS/MS.



	Formulation					
	A		B		C	
	% Dosed		% Dosed		% Dosed	
Segment	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Nares	11.36%	0.07	3.59%	0.00	19.68%	0.23
Olfactory bulb	0.00%	0.00	0.00%	0.00	0.00%	0.00
Pharynx	0.00%	0.00	0.00%	0.00	0.00%	0.00
Sinuses	0.00%	0.00	0.00%	0.00	6.96%	0.22
Turbinates	88.63%	0.07	96.41%	0.00	80.31%	0.23

Figure 9. Three formulations were tested using the same single dose delivery device on n=3 casts per formulation without extraneous airflow. Casts were prepared according to methods developed in Figure 8. Devices and casts were placed in a holding/actuating device to ensure consistent dosing, and a single dose was delivered to each nostril. The casts were disassembled and extracted using the identified method. Relative amounts of drug delivered to each analyzed region are given in figure 9 and the adjacent table.

CONCLUSION

Complexity of nasal drug delivery comes from a host of sources that are either not well controlled with development (e.g., patient compliance), or that can be de-risked to an extent with development. Development of better models helps further de-risk drug development. No model system is a perfect representation of the *in vivo* state. However, development of new model systems that are closer to the physiology of the nasal mucosa (reconstructed, differentiated epithelial tissue), and more representative of the nasal passages serve as improvements in the developing field.