Evaluation of In Vitro EpiOral™ Buccal Mucosa Model for Studying Nicotine Permeation

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Background and Purpose

In vitro models that generate reproducible insights on nicotine permeation across buccal mucosa from different formulations can be useful for oral tobacco and nicotine product development and regulatory considerations. Limited information exists in the literature on the application of EpiOral™, an in vitro 3D human buccal tissue model, for the evaluation of nicotine permeation.

The present feasibility study aimed to evaluate the suitability of the EpiOral™ model for evaluating nicotine permeation under different in vitro conditions including varying nicotine concentration and pH levels.

Methods

Test System: Human EpiOral™ (ORL-200, donor G29) oral/buccal tissues (MatTek Corporation)

Study Design: The tissues were equilibrated to 37°C overnight according to MatTek protocol prior exposure of varying clinically relevant nicotine concentrations[1] (1mM, 3mM, and 10mM) on the apical side, at pH 6.8 (~pH of human saliva) across 3 independent trials with 4 to 6 tissues per trial. The nicotine permeating to the receiver/basolateral side was measured at regular intervals for up to 240 min and used to calculate the cumulative permeation and apparent permeation coefficient (Papp in 10^{-6} cm/s).

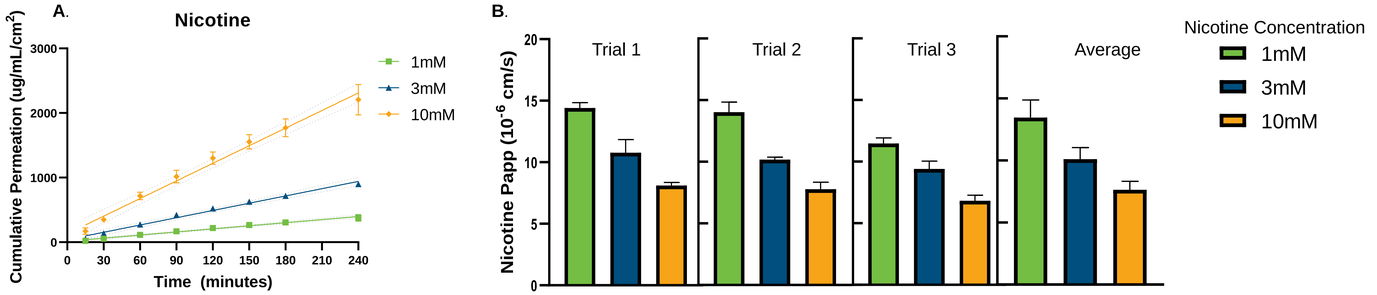
The potential effect of pH were investigated at 3 mM nicotine at pH 6.8 and 8.5. Control chemicals (atenolol (slow) and caffeine (fast)) were also tested to confirm the model's ability to differentiate between substances with different permeation kinetics. Treatment-related barrier integrity was evaluated post-treatment using Lucifer Yellow (LY) permeation and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) viability assay. Triton X-100 at 1% was used as a positive control for tissue integrity.

Test Materials & Conditions	Purpose
Nicotine (1, 3 and 10mM)	Test article for Permeability
3mM Nicotine at pH 6.8 and 8.5	Evaluate Effect of pH on Nicotine Permeation
Nicotine (1,3 and 10mM) + 1% Triton X-100	Positive Control for Tissue Integrity Disruption
Nicotine (1, 3 and 10mM) + Cell Free Inserts	No Tissue Controls
Caffeine (50µM)	Rapid Permeation Control
Atenolol (100µM)	Slow Permeation Control

End Point	Purpose
Cumulative Permeation	Used to determine linear range of x (time) and y (change in the receiver nicotine concentration) for Papp calculations
Permeation Apparent (Papp)	Permeability Measure in cm/s $P_{app} = (dC_r/dt) \times V_r / (A \times C_0)$: expressed as the apparent change in receiver concentration of the analyte over time related to the receiver compartment volume, normalized to surface area and donor analyte concentration at time zero
Lucifer Yellow (LY)	Barrier Integrity Endpoint Low LY Papp = Stronger Barrier
MTT	Cell Viability (toxicity) Endpoint: Low MTT = Less viable cells; compromised barrier (toxicity)

Results

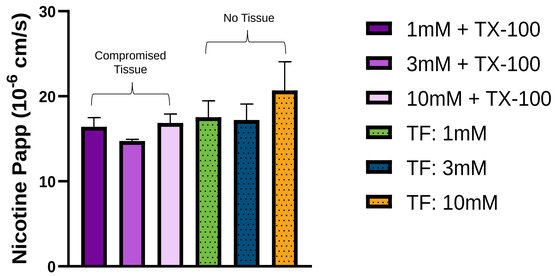
Figure 1. Nicotine Permeation. A. Cumulative permeation of nicotine at pH 6.8, over 240 minutes. The data is mean ± standard deviation of 3 independent studies, each independent study may have up to 6 replicates. Dotted lines indicate the 95% confidence interval of the linear regression. **B.** Nicotine permeation (Papp) over a range of nicotine concentrations at pH 6.8. Data is shown from three independent studies, as well as average of independent runs.



- Cumulative permeation of nicotine consistently and linearly increased with increasing at all concentrations.
- A concentration-dependent decrease in permeation was observed for nicotine at pH 6.8 (Papp-10^{-6} cm/s 1mM: 13.5±1.4, 3mM:10.1±0.92 and 10 mM: 7.6±0.67).
- This concentration dependent decrease in Papp of nicotine with increasing concentration has also been observed in this tissue model previously[2] as well as in other models (TR146 cell line and porcine buccal tissues[3]).
- The permeation of nicotine was reproducible across independent studies with both inter and intra-assay variability of <10%.

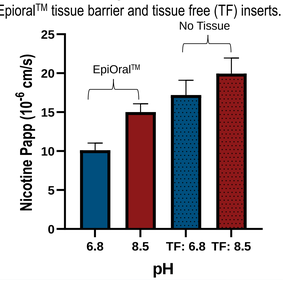
EpiOral™ demonstrated reproducible nicotine permeation, with expected concentration and pH effects observed, suggesting suitability as an in vitro model for the study of nicotine permeation.

Figure 2. Role of Tissue Barrier on Nicotine Permeation. Nicotine Papp in Triton X-100 (TX-100) treated EpiOral™ and tissue free (TF) cell inserts.



- In absence of tissue barrier there is no notable difference in nicotine Papp across all concentrations, as previously noted in the presence of uncompromised barrier (Figure 1B).
- Nicotine permeation was substantially higher in cell inserts with compromised barrier (Triton-X, Papp ranges 14.7-16.9) and inserts without barrier (TF, Papp ranges 17.5-20.7) relative to the nicotine permeation in presence of uncompromised barrier (Figure 1B)

Figure 3. Effect of pH on Nicotine Permeation. Nicotine permeation (Papp) at 3mM and pH 6.8 and 8.5 in inserts with Epioral™ tissue barrier and tissue free (TF) inserts. Data is shown as average from three independent studies.



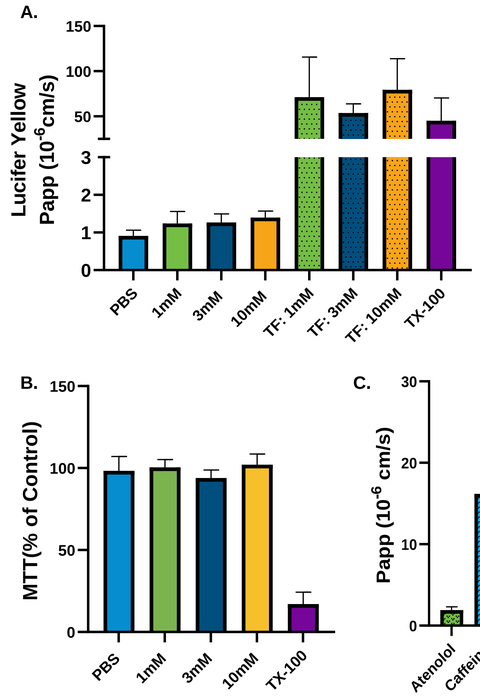
- Nicotine permeation increased with increasing pH: the increase is likely associated with an increase in the nonionized form of nicotine[3] at pH 8.5 compared to pH 6.8.
- The resulting Papp increased from 10.1±0.92 at pH 6.8 to 15.0±1.07 at pH 8.5 (from 3 independent trials) whereas minimal pH effects were observed in tissue free inserts.

Strengths & Limitations

Strengths: 1) Use of more clinically relevant model, EpiOral™ which is human derived with in vivo-like 3D morphology; 2) The model was able to differentiate between fast and slow permeants, show effects of pH and concentration; 3) Incorporation of barrier integrity and cell viability in each study allowed the ability to differentiate between true permeation and permeation due to lost barrier; 4) High reproducibility across all endpoints was observed.

Limitations:1) Donor variability was not assessed; 2) The duration of exposure was exaggerated related to known product use observations (240 mins vs. 15-20 mins); 3) The model may not be appropriate to predict in vivo uptake as in vitro-in vivo correlations have not been established. However, it may be useful for screening of relative changes in permeation.

Figure 4. Assay Controls. A. Permeation of Lucifer Yellow (LY), tissue integrity marker ; B. MTT assay for cell viability. C. Slow (atenolol) and fast (caffeine) permeates.



- Loss of barrier integrity (LY Papp <2) and cytotoxicity were not observed at any of the nicotine concentrations evaluated. In contrast, tissues treated with Triton X-100 showed increases in cytotoxicity (increased LY and decreased viability (<12%) (TX-100 1hr treatment shown as representative).
- The model was also able to differentiate between slow (Atenolol: Papp = 1.91±0.39) and fast permeant (Caffeine: Papp = 16.2±0.87) which were highly reproducible within and between repeat studies.

Conclusion

Overall, the EpiOral™ model demonstrated reproducible nicotine permeation under various conditions, suggesting its suitability as an in vitro model for the study of nicotine permeation.

References

1. Holliday R.S. et al., Journal of Dentistry 86 (2019) 81-88
2. Keyser B.M. et al., Toxicology Letters 392 (2023) 36-45
3. H.M. Nielsen, M.R. Rassing, European Journal of Pharmaceutical Sciences 16 (2002) 151 –157

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