Characterisation of an in vitro air-liquid interface (ALI) inhalation exposure system.

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Introduction

In vitro inhalation exposure systems have emerged as popular potential solutions for studying toxicological effects of aerosols. These systems are becoming increasingly complex in their function. The VITROCELL® Cloud Alpha MOVE features a quartz crystal microbalance (QCM) for dosimetry, and a sequential dosing mode which uncovers wells during the experiment to study dose-response relationships. It is important that characterisation and validation of new systems is performed prior to study design and first use.

Aims

- Validate the VITROCELL® Cloud Alpha MOVE.
- Ensure it meets the required quality and safety standards.
- Work up procedures for integration into a research project.

Methods

Using an Aerogen® vibrating mesh nebuliser (AG-AL1000; MMAD 4.0-6.0 µm), 20 µg/ml sodium fluorescein was aerosolised into the cloud generation chamber. The aerosol was allowed to gravitationally settle into metal inserts containing 100µl PBS acting as trapping liquid. A sample from each metal insert was fluorometrically analysed and compared to a standard curve to determine the concentration of sodium fluorescein. This method was repeated using the single exposure function, where all wells were exposed once, and the sequential dosing mode, where columns one to six were exposed five to zero times respectively.

Values were compared between each well and between wells and QCM readings to determine variability in aerosol deposition. Data was analysed via paired t-test, two-way ANOVA, and descriptive statistics.

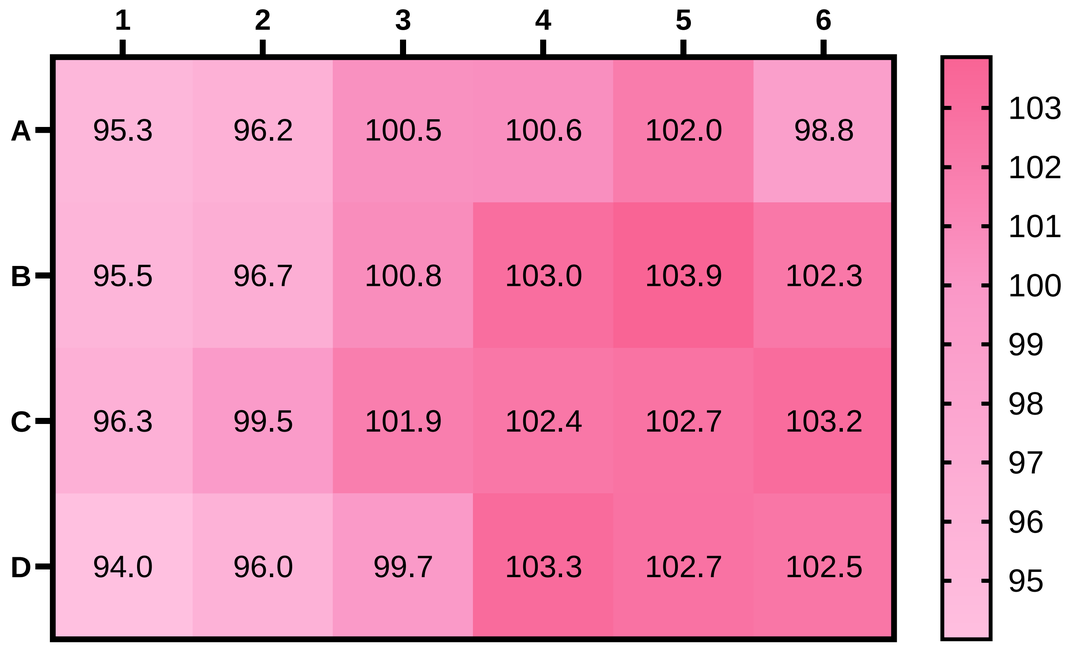

Figure 1. Average deposition of aerosolised sodium fluorescein in each well of the Vitrocell Cloud Alpha MOVE as a percentage of the mean deposition. Each well was sampled after a single 500 µl aerosolisation of 20 µg/ml sodium fluorescein (N=6). The coefficient of variation across all wells is 3%.

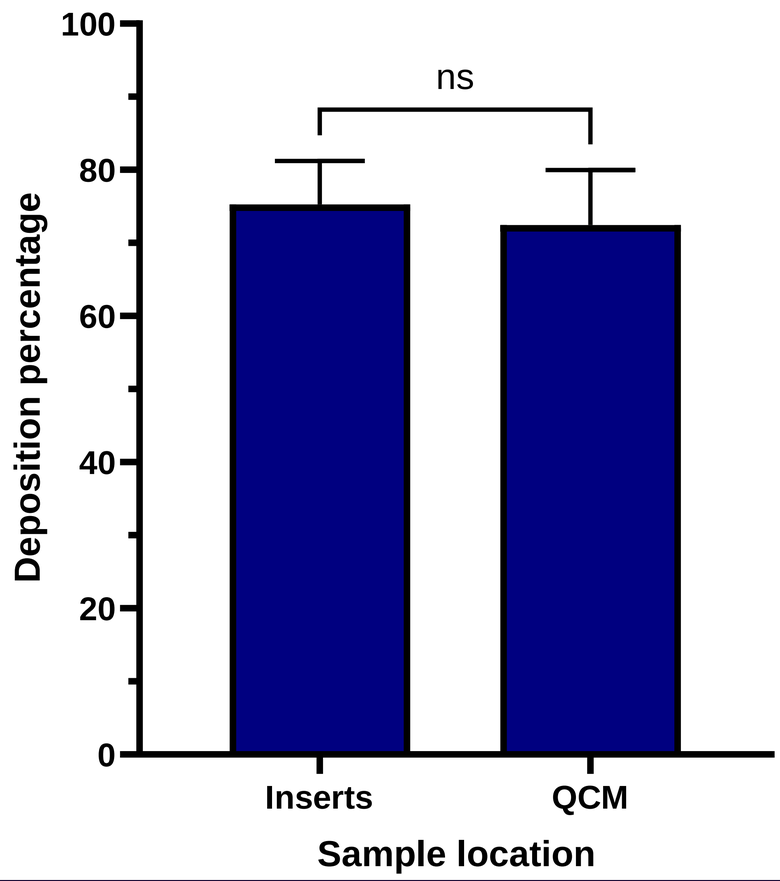

Figure 2. Deposited sodium fluorescein as a percentage of maximum possible deposition. Samples were collected from inserts and then compared to the quartz crystal microbalance (QCM) reading (N=9). Mean deposition % for the inserts and QCM was 75.3% and 72.4% respectively, and shows no statistically significant difference (paired t-test; p>0.05).

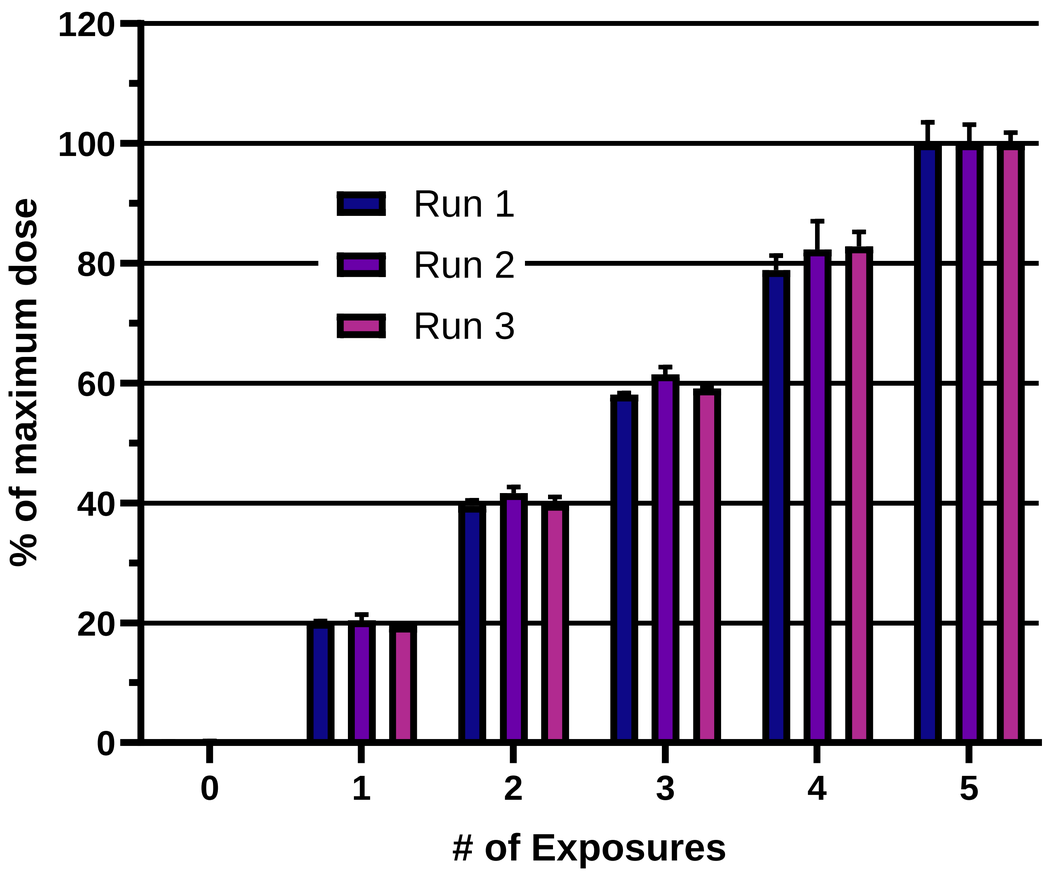

Figure 3. Sodium fluorescein doses delivered to wells after receiving zero to five exposures during the sequential dosing mode (N=3). The doses were normalised to the total cumulative dose received by an average well after 5 exposures.

Results

- Variation of the delivered dose between inserts is ±6% of the mean after all wells are exposed to a single dose of aerosolised sodium fluorescein. The coefficient of variation of delivered doses between inserts is 3%.
- On average, the deposition of sodium fluorescein to the inserts was 75.3% of the maximum. The average deposition to the QCM was 72.4%. There was no statistical difference between the inserts and QCM deposition percentages.
- When using the sequential dosing mode, each subsequent nebulisation of sodium fluorescein increased the total dose received by the inserts in a stepwise manner. Inserts received doses increasing in 20% increments, normalised to the maximum dose received by inserts, over the course of 5 nebulisations.

Conclusions

- There is very low variation in the deposition of aerosol between wells over a single nebulisation. Therefore the aerosol deposition can be considered equal across all wells.
- Variation between wells could be due to slight difference in nebuliser performance between nebulisations.
- The insert and QCM deposition percentages were not statistically different, meaning that the QCM can be reliably used to determine the dose delivered to each insert.
- The sequential dosing mode increased the dose delivered to inserts in a stepwise manner after each aerosolisation. This means that the repeated nebulisations are consistent, the sequential dosing mode can be used to perform dose response experiments, and the doses delivered using this function can be accurately calculated.

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