Development of a prototype of an aerosolization device for dry powders to improve *in vitro* cell-based assays in the context of lung delivery

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Summary

The lung has been, for the past years, subject of intense research for local and systemic drug delivery approaches. However, robust correlations between *in vitro* and *in vivo* results are often impaired, as *in vitro* experiments frequently have limitations mimicking lung conditions, especially when involving cell-based studies. This work proposes a 3D-printed device for aerosolization of dry powders over cells in culture, thus better resembling the conditions of aerosolization occurring *in vivo*. The proposed device comprises two parts, the weighing head, and the main body. Dry powders are weighed in the weighing head, which is then sealed to the main body. Next, compressed air is injected into the device, dragging the powder onto a plate, which fits the bottom of the main body. The plate is a suitable surface for cell culture. Dry powders (polysaccharide-based microparticles) were tested using the device, leading to an aerosolization yield up to 51%, which was observed to depend on the tested dry powder. The dry powder deposition profile is currently being evaluated using a Quartz-Crystal Microbalance, which replaces the plate at the bottom of the device's main body. These experiments entail a more precise determination of the mass of aerosolized powders by analysing the differences in the vibration frequencies of a quartz crystal, thus allowing to assess the deposition profile. Although preliminary, the results show that the developed device may comprise an affordable solution for *in vitro* testing of dry powders when aerosolization over a surface is required.

Key Message

The developed prototype of an aerosolization device can homogenously disperse dry powders over cell layers, comprising a cheaper alternative for *in vitro* cell-based experiments that allows better resembling the conditions of powder aerosolization.

Introduction

The paradigm of drug administration has been changing for the past years, as alternative routes, new technologies, and strategies have been widely studied to improve therapeutic outcomes.[1,2] Lung drug delivery has been considered a relevant alternative for the treatment of local diseases and for the systemic delivery of drugs. Nevertheless, the approach requires complying with aerodynamic requisites to reach the appropriate zone of the lung.[3] Additionally, different regions of the respiratory tract present different physiological environments: the bronchi have a mucus layer, while the alveoli are completely devoid of liquid, being covered only by a thin layer of lung lining fluid preventing desiccation[4]. This contrasts with more conventional routes, such as the oral, characterised by abundant volume of liquid surrounding the mucosal tissue, making the evaluation of behaviours of inhalable drug formulations challenging. *In vitro* tests comprise the first line of assessment to ascertain the potential of drug formulations and are expected to provide robust and indicative information on the formulation performance. Dry powders are currently of the most popular inhalable formulations, but their evaluation in cell-based assays is complex, given the difficulties in mimicking the lung environment. Conventional cell-based experiments involve considerable volume of liquid, in which cells are cultured with liquid on the apical side. Dry powders are often described to be simply dispersed in the liquid, which does not resemble *in vivo* conditions in the specific case of the lung route. Delivering dry powders as such is, in fact, a difficult task. The market provides some adequate solutions to tackle the *in vitro* testing of dry powders, all involving quite complex and expensive equipment not easily accessible to most of the inhalation community. That limitation impelled us to design and develop an easy-to-use and economically accessible device that allows the aerosolization of dry powders, providing homogenous dispersion over a plate, intended to have cultured cells at a later stage. The device targets initial stages of formulation development frequently impaired by the inability to afford high-cost equipment. Its use is expected to allow refining and selecting drug formulations for more advanced stages. The proposed device has much lower cost in comparison with other alternatives in the market and it can have broader application related to the evaluation of the impact of pollution or electronic cigarettes in the human respiratory system.

In this work, a device for dry powder aerosolization onto a plate, which is suitable for cell culture, was developed and the protype printed in 3D. Polysaccharide-based unloaded microparticles prepared by spray-drying were used to test the device. The yield of the aerosolization process was calculated and air flow parameters are currently being optimised using a quartz crystal microbalance (QCM),[5] which will enable subsequent assessment of the deposition profile of selected dry powders. The proposed work is summarized in Figure 1.

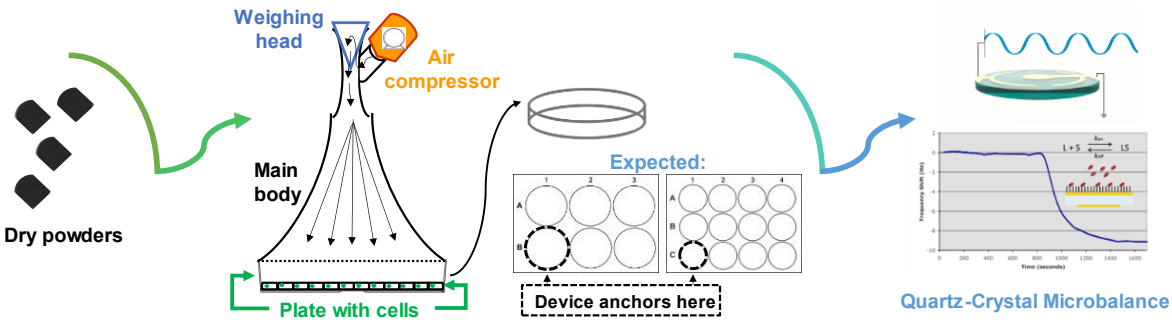


Figure 1 – The concept supporting the design and development of a prototype of an aerosolization device to be used in *in vitro* cell-based assays. From left to right, dry powders are weighed and loaded into a weighing head (in blue) that is subsequently assembled with the main body of the device. Compressed air is then loaded into the device (orange), dragging the powder (arrows), and dispersing it over cells cultured in a plate. The dry powder deposition profile is further analysed using a quartz crystal microbalance, through differences in the crystal vibration frequency.

Experimental methods

Design and printing process

The device was conceived and designed using a commercial software (AutoCAD, Autodesk, Inc., USA) and exported to a commercial 3D printer (ULTIMAKER 3 Extended®), equipped with a standard 0.4 mm diameter double extruder nozzle but used in single mode. The apparatus was comprised of an extrusion system and a heated printing bed, which works synergistically with the extruded polymer (in filament form of 2.85 mm) at high temperatures. Printing was performed at 225 ºC using biodegradable poly-lactic acid. The nozzle height was set at 1.0 mm, with layers of 0.2 mm being applied. The extrusion speed was set at 17 mm/s.

The design and printing of the prototype considered two pieces, one comprising a small weighing head, where the dry powder is to be weighed, and another as the main body, conceived to allow powder dragging and deposition over cell layers.

Aerosolization of powders

Polysaccharide-based (unloaded) microparticles previously developed by the team for lung applications, composed of xanthan gum and dextran sulfate, and produced by spray-drying (Buchi Mini Spray-Dryer, Buchi Labortechnik AG, Switzerland), were selected as model dry powders to test the device. Before the assay, all parts of the device and a plate suitable for cell culture were weighed. Afterwards, 5 - 10 mg of dry powder were weighed in the weighing head, the device assembled, and the powder aerosolized after blowing air into the device (30 quick puffs from an air compressor, Goldair). The outlet pressure of the air compressor varied between 30 and 60 psi. After conclusion of the assay, the parts of the device and the plate were weighed, and images of the plate recorded after powder deposition. The yield of aerosolization was calculated as follows:

Yield of aerosolization (%) = (Amount of powder on the plate / Total amount of powder weighed) * 100

Evaluation of the profile of dry powder deposition by QCM

Maxtek quartz crystals (5MHz, Inficon), coated with optically flat polished gold electrodes on both sides, were cleaned before use with absolute ethanol, ultrapure water and a Piranha solution comprised of 3:1 mixture of sulphuric acid and 30% hydrogen peroxide for 15 min. Cleaned crystals were mounted on a kynar crystal holder (Maxtek CHT-100). The 3D-printed device was placed over it, and the aerosolization assay performed as described above. Changes in the vibration frequency of the quartz crystal were registered with a universal frequency counter (Agilent 53131A) through a phase-locked oscillator (Maxtek PLO-10i). The frequency counter is connected by GPIB to a computer running a data acquisition and control program developed in VEE V9.0 (Agilent) for the real time acquisition of the QCM frequency.

Results and discussion

Following the concept proposed in Figure 1, the device shown in Figure 2 was designed and prepared using the software AutoCAD. The final 3D-printed prototype is shown in Figure 3.

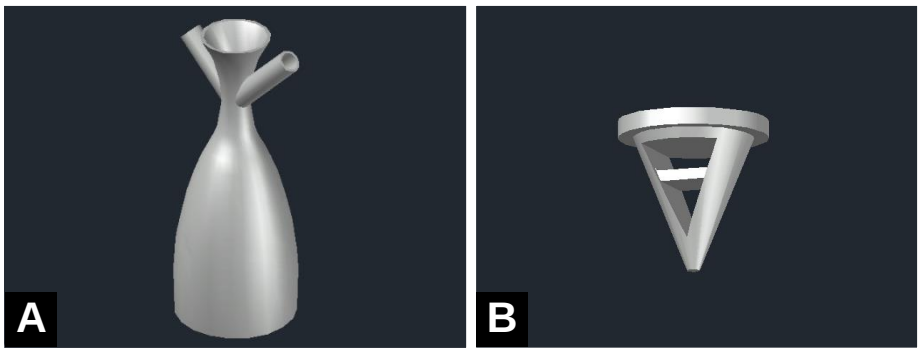


Figure 2 – AUTOCAD design for the main body (A) and the weighing head (B); the powder is weighed in the latter.

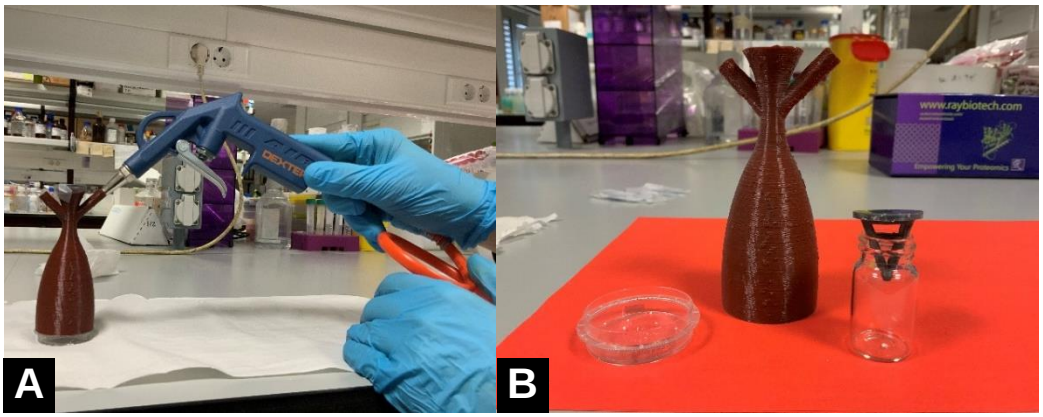


Figure 3 – 3D-printed prototype of the device and its use in the aerosolization assays: (A) the device assembled and prepared for an assay and (B) the different parts of the device, after an assay.

One of the limiting steps of the aerosolization process is the strength of the applied air flow, which should be enough to disperse all the powder but not excessive to induce destabilisation of the cell layer. In the first stages of development, various sources were tested for blowing air: lens cleaner, syringe, bicycle pump and a gardening sprayer. The latter performed better, but it was concluded that the strength of the air flow was not enough to adequately disperse dry powders onto the plate. Therefore, an air compressor was tested to enhance the amount of dispersed powder. The aerosolization protocol was optimised, establishing a quick blow of air 30 times with an air blow gun (Figure 3A) as the most effective way to ensure that all the powder is dragged to the plate.

The following step involved determining the ideal operating air pressure to be set in the air compressor, aiming at two objectives: 1) maximise the amount of dispersed powder and 2) generate enough flow intensity without forcing powder dispersion to outside of the testing device. Various intensities of air pressure were tested to aerosolize different dry powders, all developed for inhalation purposes, and results are depicted in Figure 4.

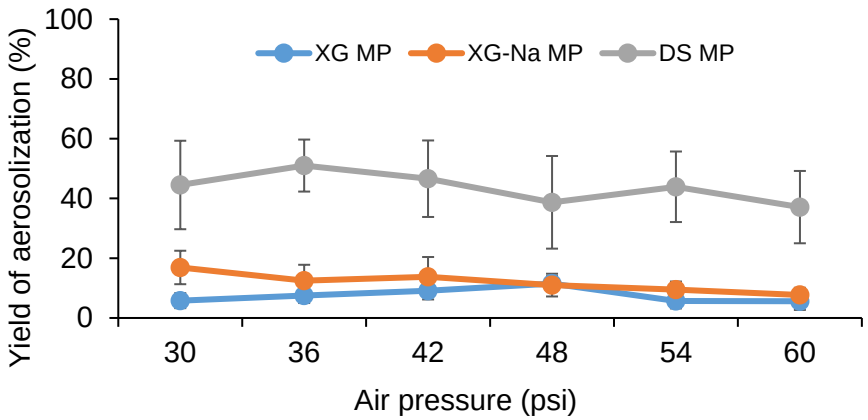


Figure 4 – Aerosolization yields obtained with the device prototype, for microparticles (MP) composed of xanthan gum (XG), xanthan gum/sodium chloride (XG-Na) and dextran sulfate (DS). Results are presented as mean ± SD, n = 10.

The obtained results indicate that pressures of 36 and 42 psi are those maximising the yield of aerosolization, that is, the amount of powder leaving the weighing head onto the plate. Observations of tests performed at different air pressure indicated that dry powders comprised of particles with lower tenuity have facilitated dispersion, compared with finer particles. Other polysaccharide-based microparticles are currently being tested, but results obtained so far indicate the need to optimise the device to improve the dispersion of finer particles and adapt the device to all types of dry powders.

Optimizing the air flow that goes into the device is critical for the quality of the aerosolization process and powder deposition. QCM can be very helpful in this regard as, unlike a conventional balance, it allows following the deposition in real-time with great accuracy.[5] In this way, the mass deposition *per* air blow can be followed to further optimize the procedure. To this end, the developed device was assembled on top of a QCM, which is replacing the plate, to determine the deposition profile of aerosolized dry powders at different air flow pressures. The assay is currently being performed.

Conclusion

A functional prototype of an aerosolization device able to disperse dry powders onto a plate was designed and 3D printed successfully, using a biodegradable/biocompatible polymer. The aerosolization required using an air compressor and protocol optimisation established the need to perform 30 quick air blows to maximise powder dispersion. Moreover, testing different air pressures resulted in the indication of optimal air pressures at 36 and 42 psi, which provide higher aerosolization yield. Yields up to 51% were obtained, which were observed to depend on particle tenuity, with lower tenuity facilitating the dispersion process. To further optimise the process, the deposition profile is being studied using QCM, a highly accurate balance enabling more precise and real-time determinations.

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