ARTIFICIAL NEURAL NETWORKS & TISSUE MODELS FOR INHALED FORMULATION SCREENING, SELECTION & BIOEQUIVALENCE TESTING

In this article, MedPharm CSO and COO Professor Marc Brown, PhD, CChem, FRSC; Ms Jo Muddle, PhD Student, Kings College London and Scientist at MedPharm; and Professor Clive Page, PhD, Professor of Pharmacology at King's College London, and Head of Sackler Institute of Pulmonary Pharmacology, describe the use of artificial neural networks (ANNs) and novel tissue models as methods for formulation candidate screening, selection and characterisation and, in generic product development, for bioequivalence testing. As lower-cost alternatives to, for example, in vivo approaches, and potentially more informed alternatives to next-generation impactor studies, the authors argue that the ANN and tissue model methods presented here could be usefully employed early in the development process.

Pulmonary and nasal drug delivery is on the rise. Innovations from pharmaceutical companies and device developers alike are helping to drive innovation in drug delivery via the inhalation route. The pulmonary delivery market is projected to be worth US$44 billion (£26 billion) by 2016 with a CAGR estimated at 14.3% over the next two years until then (BCC Research, 2012).

There have been a number of new drug advances recently. For example GSK has received US FDA approval for its new COPD product, the dual bronchodilator Anoro Ellipta (umeclidinium + vilanterol). This is predicted to be a major blockbuster for the company with projected annual sales of $3 billion to 2019, according to Thomson Reuters. This is in addition to last year's FDA approval of Breo Ellipta (vilanterol + fluticasone furoate), another COPD product, which analysts believe will achieve $2.22 billion in annual sales by 2018 (Thomson Reuters). AstraZeneca is continuing to push the boundaries with the development of a nasal vaccine for four strains of the influenza virus for nasal delivery. It won EU approval for the product at the close of 2013. Finally, MannKind's efforts to bring an inhaled insulin product to the market have had those in the field sitting up and taking notice. The list of companies whose programmes were terminated in this area is a long one, and success for MannKind may re-invigorate research efforts despite the failure of Pfizer's Exubera.

For a long time, one of the challenges of delivering medicines by the inhalation route has been ensuring patient compliance. Incorrect inhaler techniques, sub-optimal devices, and over and under use of medications are all cited as major compliance problems. Only 40-60% of COPD patients are reported to stick to the correct dosing regimen and only 10% of asthma patients perform all steps correctly when using a pMDI.[1] To combat this, device developers and manufacturers have been steadily improving the design of delivery devices. For example, installing dose-counters on pMDIs allows patients to see when they need to ask for a new inhaler, whilst improved hand-held nebulisers benefit the end-user by providing a more user friendly and convenient device.

The growing healthcare burden in many developed countries is also encouraging growth in the inhalation generics field. With a number of patent expiries looming, initiatives are in place to boost the progress of these medications to market, through changes to regulatory requirements. Recently the FDA relaxed its stance on the need for lengthy clinical trials for generic versions of the GSK product Advair (fluticasone propionate + salmeterol xinafoate) in order to show bioequivalence; an often challenging hurdle for companies. Still, the cost of bring-

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ing a generic to market may easily run into the tens of millions of dollars, thus innovations in research and development tools are required to reduce this financial burden and to encourage generic and NCE airway product development. Such innovations should also reduce the inherent risk within R&D work by mitigating the chance of later stage attrition.

ARTIFICIAL NEURAL NETWORKS

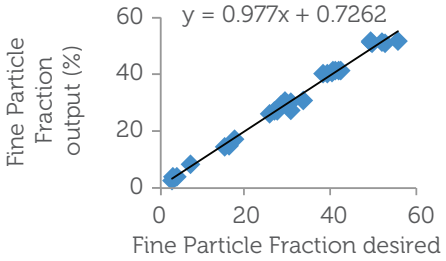
MedPharm is pioneering this effort and offers the full range of API characterisation and selection, device selection, biological testing, formulation development, *in vitro*, *in vivo*, preclinical and clinical testing services and GLP/GMP supplies to its clients to aid their developmental programmes.

Innovation in research techniques and tools is ongoing within the company and whilst techniques such as API and excipient characterisation, formulation development, and next-generation impactor (NGI) testing are routine for the company, new services are also made available to clients. For example, MedPharm has developed a new *in silico* model based on artificial neural networks (ANN) to enable early selection of the most promising APIs and formulations to take forward into testing, a technique that is already creating a stir at industry conferences.

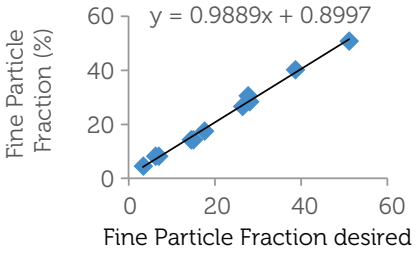
ANNs have been widely recognised as powerful pattern recognition tools in areas such as forecasting finance and medical diagnosis. In addition, ANNs have been shown to be beneficial when analysing drug delivery in the pharmaceutical science area. For example ANNs have been used to predict drug delivery to the lungs *in vivo*. Nazir and colleagues reported the use of ANNs in this context using a variety of input factors: different breathing patterns; particle size; mass median aerodynamic diameter (MMAD); and geometric standard deviation, to predict the aerosol particle deposition in the different regions of the lung.[2,3] De Matas and colleagues used ANNs to predict a variety of pharmacokinetic (PK) responses for delivering inhaled drug into human lungs using similar input variables as Nazir's studies.[4-6] Both groups showed the success of using ANNs to predict the pharmacodynamic (PD) and PK effects of delivering drug to the lungs, albeit with a dataset of limited size.

Although ANNs have been used to predict *in vivo* outcomes from *in vitro* data, ANNs have not yet been reported as a means of predicting impactor data, from an NGI for example, or the parameters that can be derived from these studies (i.e. MMAD, fine particle fraction (FPF) and emitted dose). Recently, MedPharm has been able to show the feasibility of using different formulation and device characteristics to predict drug deposition *in vitro* (Figure 1).[7] The next stage of assessing the viability of using ANNs to predict FPF will be to test a larger dataset with a variety of different DPIs and APIs.

A) Training set
Mean Squared Error – 2.300%
Normalised Mean Squared Error – 0.010%
Mean Abs Error – 1.133%
Min Abs Error – 0.044%
Max Abs Error – 4.091%
R - 0.995



B) Cross Validation set
Mean Squared Error – 1.763%
Normalised Mean Squared Error – 0.009%
Mean Abs Error – 0.976%
Min Abs Error – 0.037%
Max Abs Error – 2.989%
R - 0.997



C) Test set
Mean Squared Error – 5.190%
Normalised Mean Squared Error – 0.052%
Mean Abs Error – 1.904%
Min Abs Error – 0.233%
Max Abs Error – 4.182%
R - 0.979

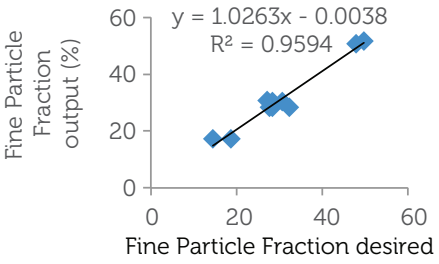


Figure 1: Errors and the R^2 value produced for the multilayer perceptron ANN for the training set, where the ANN is trained to minimise errors between the desired output and expected output from: (A) known data, (B) cross validation set, and (C) test set. "FPF desired" is the actual FPF value generated from the NGI studies. This is compared with "FPF output"; the FPF predicted by the artificial neural network.

The studies so far have been promising and have shown ANNs as a viable technique for predicting the output of NGI. In the future ANNs, with ongoing innovations and progress in this area such as those occurring at MedPharm, could be used instead of NGIs to predict the drug deposition of new inhalers, with NGIs employed only for quality control and confirmation purposes. In the nearer term, ANNs could certainly provide a method for formulation screening, prior to the commencement of costly NGI studies.

MEDPHARM CSO PROFESSOR MARC BROWN ELABORATES:

In silico modelling is a growing area of research in pharmaceutics. Many different *in silico* methods have been used to help speed up production of inhaled products, including techniques such as Box Behnken and the Taguchi method. ANNs are another prediction tool that can be used to select the best API formulation candidates to take forward into testing and optimisation, reducing costs in the already expensive process of new product development. Overall, there is a vast range of *in silico* models that can be used to speed up the process of developing a formulation and cut the risk of attrition at a later stage by eliminating poorly performing formulations before they are taken forward into more expensive and time consuming studies.

TISSUE MODELS

In addition to ANN, MedPharm is currently developing tissue models to allow PD activity of inhaled products to be examined. These models are particularly useful in the process of showing PD bioequivalence when developing a generic product. Currently, there are few bioequivalence tests that are accepted by the regulatory authorities. One of these is the NGI assay, which maps where the formulation is deposited.[8] However

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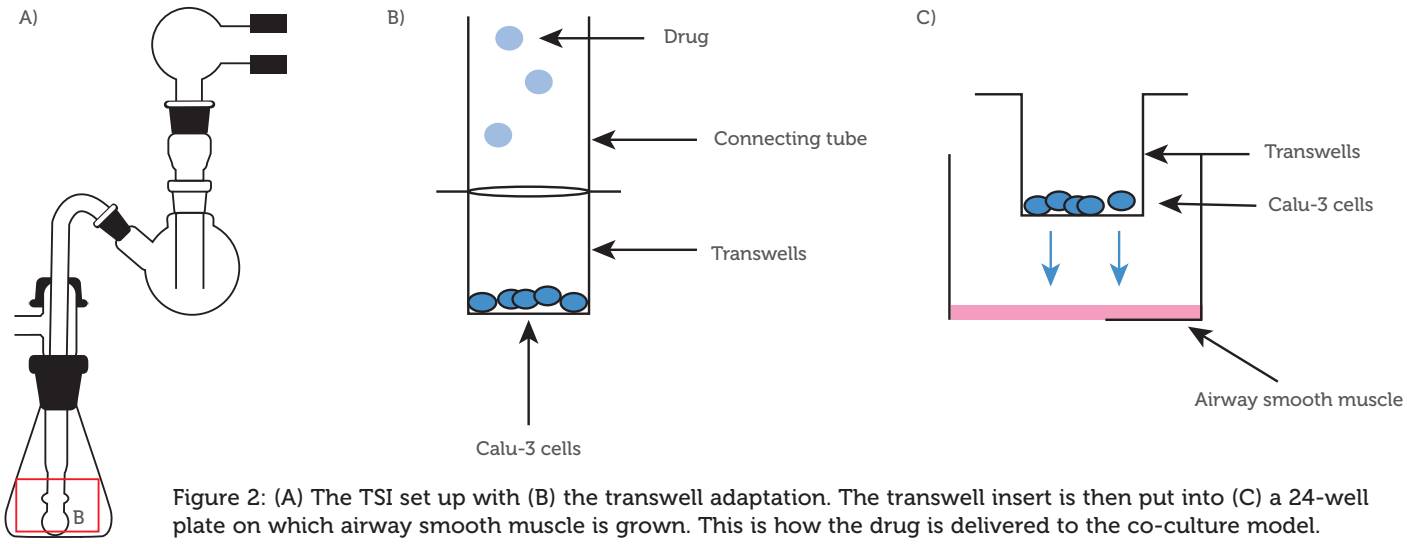

Figure 2: (A) The TSI set up with (B) the transwell adaptation. The transwell insert is then put into (C) a 24-well plate on which airway smooth muscle is grown. This is how the drug is delivered to the co-culture model.

NGIs do not examine whether the delivered formulation is biologically active at the site of deposition. At present, studies examining the PD and PK abilities of a drug are *in vivo* studies, which are often very costly and time consuming. MedPharm is providing a solution to this gap in the market that will enable its clients to generate vital data without the need for theses *in vivo* studies.

The basic model itself is a co-culture model which can be used to test bioequivalence between inhaled products. To date, work has involved using Twin stage impingers (TSIs), used to deliver potentially respirable powders with an aerodynamic diameter of less than 6.4 µm, onto Calu-3 epithelial cells grown on an air liquid interface.[9] Drug flux across the epithelial layer can then be analysed. This study has highlighted the feasibility of using this kind of method to assess formulation performance *in vitro*. Indeed, some of the FITC-dextran weights measured *in vitro* successfully correlated to *in vivo* canine pulmonary clearance. MedPharm is taking its model one step further by developing the co-culture model to allow measurement of the drugs' biological activity once absorbed. The novel model allows the delivery of the formulation, via TSI or NGI, to the Callu-3 cell epithelial layer, beneath which is a cultured layer of airway smooth muscle, allowing assessment of the PD activity of any drug that has been delivered (see Figure 2). This allows absorption, deposition and PD activity to be assessed at the same time.

In combination, all of MedPharm's models can be used to look at the efficacy and the *in vitro* performance of the inhaled formulation. This will help in candidate and formulation selection and provide a better model for bioequivalence testing. In addition, these techniques may also help to give a clearer picture of what happens in the lung without having to undertake *in vivo* studies.

Advances in R&D tools such as these, whilst perhaps not revolutionising the development process, will certainly help reduce costs and the time taken to bring a product to market. Risks can be minimised in a process early on.

Described here are just two such advances in technology; and as part of MedPharm's contract services, the company offers the full range of services needed to take inhaled, as well as API formulations for transdermal and topical (skin, nasal, ophthalmic, buccal and mucosal) delivery, through formulation development, testing and clinical trial material manufacture.

MedPharm prides itself on developing novel research tools such as *in vitro* efficacy models and *ex vivo* toxicity assays to aid its clients' programmes. With hundreds of worldwide clients, and experience of helping to bring numerous products to market, including DPIs, pMDIs and nebulisers, as well as a host of other topical and transdermal products, the company comprises a highly experienced team, leading the field. As development companies increasingly look to take advantage of strategies to reduce risk and cost, innovative contract research and manufacturing companies such as MedPharm, who can offer turn-key solutions, are in a strong position to assist.

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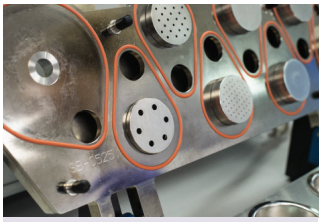

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