wildtype (Dam+) strain to kill 50% of the animals [1,4].

The next step was to test whether Dam mutants could serve as a live vaccine to protect mice against infections from wildtype *Salmonella.* The group showed that inoculating mice with Dam mutants protected against subsequent exposure to virulent wildtype strains. They also recently showed that *Salmonella* Dam– and Dam$^{OP}$ live, attenuated vaccines elicited cross-protective immunity to three different heterologous *Salmonella* serotypes in mice and chickens [1,2].

Mahan and colleagues also explored the role of Dam in the pathogenesis of two other enteric bacteria, *Vibrio cholerae* and *Yersinia pseudotuberculosis*, the causative agents of human cholera and gastroenteritis, respectively [5]. They found that Dam overproduction attenuated the virulence of both bacteria. In the case of *Y. pseudotuberculosis* it led to a fully protective immune response in vaccinated hosts. They concluded that, because mutations in Dam can attenuate the virulence of several diverse pathogens, the role of DNA methylation in virulence might emerge as a common theme in bacterial pathogenesis and could be used to elicit a class-protective immune response to more than one bacterial strain.

## Future studies

Mahan is concerned that many regulatory hurdles will need to be overcome before a license using this technology is granted for a live vaccine for human use. For this reason much of the research effort is now being directed to see if Dam technology can be used to immunize animals with killed bacteria. Mahan believes that the first use of the Dam technology in clinical trials is likely to be against tumours in cancer patients where radiotherapy and chemotherapy have failed. 'The idea would be to get *Salmonella* to express the antigens that are being made in higher quantities by cancer cells in the hope of eliciting a heightened immune response,' explains Mahan.

'The discovery by Mahan's group that dysregulation of Dam renders a variety of bacterial pathogens avirulent, yet capable of stimulating cross-protective immunity, is a significant step towards rapidly generating efficacious vaccines for several infectious diseases,' comments Brad T. Cookson, Associate Professor in the Departments of Laboratory Medicine and Microbiology at the University of Washington (Seattle, WA, USA). 'Further, by understanding how Dam mutants stimulate cross-protection, the potential of efficiently eliciting immune responses to heterologous antigens expressed by viable bacterial vectors would have important applications in medicine outside of infectious diseases.

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# Electronic DPI for insulin

**Jo Whelan**, Freelance writer

Researchers at MicroDose Technologies (Monmouth Junction, NJ, USA) have developed what they believe is the first completely electronic dry powder inhaler (DPI). The device has produced encouraging results in Phase I studies of pulmonary insulin delivery.

Millions of people with diabetes must inject themselves with insulin to control blood glucose levels. Many find this painful and inconvenient, and there have been numerous attempts to develop a non-invasive delivery system.

Insulin is a peptide and, therefore, cannot be administered orally because it would be degraded by digestive enzymes. Approaches to produce an oral formulation that bypasses this problem are being investigated, as are transdermal, buccal and nasal delivery. However, there is general agreement that pulmonary insulin delivery is viable [1]. Several inhaled insulin products are in clinical trials and the first are expected to reach the market within two years.

## Pulmonary delivery

To be absorbed effectively, the insulin dose must be carried deep into the lungs to the alveoli. The problem is that particles deposited further up in the airway will not reach the bloodstream. Particle size is crucial for deep lung penetration and, therefore, efficacy. The insulin that is fused with the MicroDose inhaler is formulated by Elan Drug Delivery (Nottingham, UK), and is being developed through a joint venture by the companies called QDose. 'Pulmonary

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insulin delivery requires a particle diameter of 3.3 μm or less,' explains Scott Fleming, MicroDose's Vice President of Marketing. This is achieved using a proprietary spray-drying process, and the insulin powder is dispensed into blisters. For improved stability the insulin can be formulated with trehalose, a sugar-derivative that occurs naturally in plants and other organisms.

The MicroDose inhaler uses a high frequency piezo vibrator to deaggregate the powder into its primary particles while still in the blister (Fig. 1). The fine particles circulate to the top of the blister and are forced out into the airstream through pierced holes. They are then carried into the lung by the user's inspiratory effort. The device is breath-actuated, with a built-in flow sensor that releases the drug only when breathing is optimal. 'What is novel is that we use external energy to deaggregate the powder independently of the inhalation flow rate,' says Fleming. 'This achieves flow-rate independence at much lower cost than other devices – the unit manufacturing cost is US$3. The inhaler is smart: we can use feedback elements to create patient compliance features without software, which removes the need for software validation.' *In vitro* studies have demonstrated 'excellent' dose reproducibility (MicroDose Technologies, unpublished data).

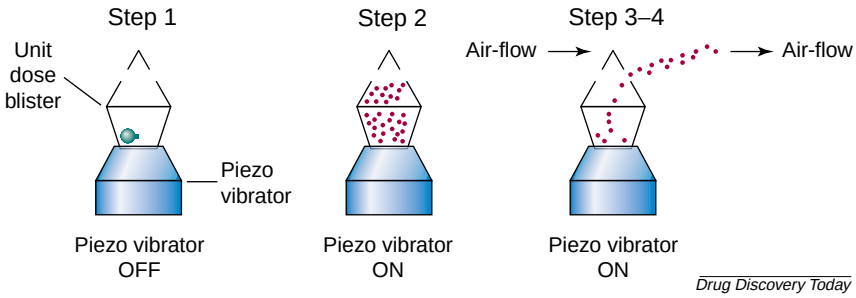


**Figure 1.** Principle of operation of the MicroDose dry powder inhaler. In step 1, the piezo vibrator is off and the drug is aggregated in the blister. In step 2, the high frequency piezo vibrator deaggregates the powder into its primary particles while still in the blister. In step 3, the deaggregated particles circulate to the top of the blister where they are forced out through pierced holes into the airstream. In step 4, the user's breath carries the drug powder to the lungs for absorption.

## Clinical trials

In a Phase I trial, 18 healthy volunteers inhaled either pure insulin or insulin and trehalose. Blood levels of insulin and glucose were measured over time following inhalation and compared with those obtained after a conventional subcutaneous insulin injection, which was given on a different day.

There was rapid absorption of the inhaled insulin, with a time to maximum blood concentration ($T_{max}$) of 37–39 min depending on the formulation. The $T_{max}$ value for the subcutaneous insulin control was 102 min. The bioavailability over the initial three hours of the inhaled insulin to the subcutaneous dose was 23–25%. 'These data compare favourably with published data from competitor approaches,' says Fleming.

MicroDose CEO Anand Gumaste, a co-inventor of the technology, acknowledges that the market for inhaled insulin products will be highly competitive. Patient preference will be a key factor in the success of any device. 'Our product is pocket-sized, unlike some of its competitors,' says Gumaste. 'It is also highly efficient – almost 85% of the insulin load is emitted from the device, so we think less insulin will be needed than with some devices, which will keep costs down.' He is also optimistic that it will be storable at room temperature, unlike many insulin formulations which need to be refrigerated.

## Future aims

Like other inhaled insulins, the MicroDose product is designed to control meal-related glucose levels. Users will still need to inject a long-acting basal insulin dose each evening. Elan is researching trehalose derivatives with the potential to create a controlled release formulation to replace the basal injection.

MicroDose and Elan are currently seeking a development partner and no date has yet been set for Phase II studies. MicroDose will also evaluate the device with other inhalable drugs.

'Inhaled insulin is an extremely exciting area of research,' says Mairi Benson of Diabetes UK. 'However, we have to be sure people are getting very accurate doses. We also need to know that there are no side effects from any insulin remaining in the lungs and airways. One trial has raised possible problems with pulmonary fibrosis, and we need further reassurance about this. The MicroDose development is extremely interesting because it is essential that insulin inhalers are portable and discreet to use.

## Reference

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