EXPERT OPINION

Novel non-invasive methods of insulin delivery

Sandra Soares, Ana Costa & Bruno Sarmento†
†*CICS, Department of Pharmaceutical Sciences, Instituto Superior de Ciências da Saúde-Norte, CESPU, Gandra, Portugal*

Introduction: Insulin has usually been administered subcutaneously in the treatment of diabetes mellitus. Alternative delivery routes of insulin are expected to overcome some limitations, mainly concerned with the possibility of hypoglycemia episodes, weight gain and inadequate post-meal glucose control, in order to lead a better patient compliance.

Areas covered: This review article covers all the most relevant non-invasive insulin delivery methods under development, respective technology and clinical data available according to their status of development. Special focus is given to the systems with late clinical trial evidences, their achievements and pitfalls. Pulmonary and oral appear to be the most advantageous routes, with regard to the long list of potentially marketed products.

Expert opinion: Alternative insulin delivery to the subcutaneous administration is more and more close to the success, being fundamental that any optimized technology could overcome the overall low mucosal bioavailability of insulin, mostly due to its early degradation before absorption, inactivation and digestion by proteolytic enzymes and poor permeability across mucosal epithelium because of its high molecular weight and lack of lipophilicity.

Keywords: bioavailability, buccal, clinical trials, insulin, nasal, non-invasive, oral, pulmonary, transdermal

Expert Opin. Drug Deliv. (2012) 9(12):1539-1558

1. Introduction

The subcutaneous (SC) injections of insulin have been considered the main treatment for insulin replacement after its discovery, however, there are several barriers related to weak patient compliance and difficult accurate glycemic control that offer an extended resistance to diabetes mellitus (DM) therapy, for both patient and healthcare providers. Currently available insulin treatment options still present several limitations including the risk of severe hypoglycemia, the likelihood of weight gain, inadequate post-meal glucose control, the need for complex titration of insulin doses in connection with meals and the need for daily injections. Even newer preparations, such as injected rapid-acting insulin (RAI) analogs, have not mimicked the natural time-action profile of insulin normally seen in healthy individuals, which is characterized by an early phase insulin response and appropriate duration of metabolic effect [1,2].

Therefore, a continuous progress of innovative and non-invasive insulin delivery systems has been reached. At this moment, these systems can be found at all stages of clinical development, and promise to facilitate earlier initiation and optimization of insulin therapy for achieving better treatment outcomes. In this review will be represented an overview of the most relevant non-invasive insulin delivery methods under development, respective technology and clinical data available according their status of development.

10.1517/17425247.2012.737779 © 2012 Informa UK, Ltd. ISSN 1742-5247

Article highlights.

- Long-term safety of inhaled insulin is currently guaranteed by numerous clinical trials, including a 4-year study. Despite the postponement of the development of several inhaled insulin products, a new product will take a place in diabetes therapeutic arsenal in few months.
- Needle-free insulin delivery systems under current development are viable alternatives to injected insulin and have reached comparable clinical efficacy. Additionally, some of these systems revealed protective action against adverse effects of the insulin therapy.
- In order to overcome the low oral bioavailability of insulin, several strategies were devised, such as the use of enteric capsules/tablets, absorption enhancers, enzyme inhibitors or even insulin chemical modifications. New carriers, like HDV-I or carriers which employ vitamin B12, have been studied for the same purpose.
- Microneedles have the ability to penetrate only in stratum corneum, without reaching the nerve endings present in the dermis, creating microchannels where macromolecules are transported. As that system is combined with transdermal patches, they not only offer a continuous and controlled drug delivery, but also allow easy handling by the patient and require minimal healthcare support.
- The buccal mucosa offers a good route for insulin delivery, without gastrointestinal degradation or hepatic metabolism. One buccal insulin spray product is already being marketed in some countries; however, a new system is under development which will deliver insulin-passivated nanoparticles by means of buccal soluble film.

This box summarizes key points contained in the article.

2. Pulmonary delivery systems

The history of pulmonary insulin delivery has been a miscellaneous of hope and disappointments in the past 6 years, since the first inhaled insulin product, Exubera® (Exu) (Nektar Therapeutics, Pfizer and Sanofi-Aventis), appeared in the market. Exu was available from January 2006 to October 2007, and due to reduced sales and low acceptance by doctors and patients, Pfizer decided to discontinue its production.

It seems like that decision shuddered other inhaled insulin products being developed, such as AERx® iDMs (AERx insulin diabetes management system, Novo Nordisk and Aradigm Corp.) and AIR® insulin (Alkermes and Elil Lilly), because few months later (January and March 2008, respectively) their Phase III clinical trials were terminated by the respective companies. In fact, practical and pharmacologic properties of these products were similar to Exu, showing any evidence of clinical advantages or convenience benefits that could overcome current insulin therapies. Hence, weak adherence and low cost-effectiveness ratio of these inhaled insulin products were predictable and did not justify further investment by the companies. Nevertheless, a number of long-term studies with Exu have been performed but almost all were suddenly stopped when commercial inability of the novel product was known and Pfizer decided to return the Exu rights to Nektar, which didn't find a new marketing partner.

Fortunately, one company, MannKind Corp., kept actively the development of its inhaled insulin system, Afrezza®, which might get a place in the therapeutic arsenal in 2013. Studies with Afrezza have shown that it is different from all other prandial insulin products because it provides a unique pharmacokinetic (PK) and pharmacodynamic (PD) profile with corresponding clinical benefits, as well as a number of device characteristics that appear to mitigate problems identified with earlier technologies.

2.1 Afrezza

Afrezza consists of an innovative inhaled drug delivery platform based on Technosphere® dry-powder formulation and Dreamboat™ inhaler device completely developed by MannKind. The company had submitted a new drug application to the Food and Drug Administration (FDA) on March 2009 and it is being expected a market approval. Meanwhile, technical issues relating to next-generation inhaler, clinical utility and labeling are being addressed [3]. The formulation technology is based on a novel excipient termed fumaryl diketopiperazine (FDKP), an inert compound highly soluble in water at neutral-basic pH. Through an acid-induced reaction, FDKP crystallizes in microcrystalline plates that self-assemble into microspheres called Technosphere particles. Regular human insulin (RHI) in solution is adsorbed onto these highly porous (approx. 70%) and slightly positively charged microparticles, and the resultant Technosphere insulin (TI) suspension is flash frozen into pellets and lyophilized. The dry powder obtained is composed of 1:9 ratio of insulin/FDKP with residual amounts of water and polysorbate 80, and it is packaged into a single-dose cartridge [4].

Several strategic factors provide an efficient insulin transport of the TI particles into the deep lung. First, TI powder is characterized by low density and lack of flowability being easily dispersed due to suitable size average of 2 – 3.3 μm presented by the particles, where more than 90% of the particles have a diameter between 0.5 and 5.7 μm [5,6]. Particle size is fixed during particle formation eliminating the need of further processing such as milling, sizing or blending [7], which is an important step because additional sources of stress that could interfere with protein stability are avoided. When in contact with the interstitial fluid of the lung, TI microparticles dissolve immediately due to the large surface area of the particles and high solubility of the FDKP at physiologic pH. FDKP plays an essential role in this technology because it will indirectly act as an absorption enhancer [8,9]. Another key feature is based on the fact that insulin administered in Afrezza should be in a monomeric state, allowing a rapid absorption, even faster than RAI [10].

The MannKind's inhaler device was designed attending numerous patient-friendly properties. It is a light, compact,

high-resistance, discrete, re-usable and easy-to-use device. In addition, it can be loaded with a pre-metered single-dose cartridge with 2.5 – 10 mg of powder, which is discharged through a simple patient inhalation by the device mouthpiece [7]. FDA had requested additional studies to demonstrate equivalence of the future commercial inhaler to the first version of the device, MedTone™, which was primarily used in clinical trials. The first-generation inhaler experienced some design changes after the initiation of Phase III clinical trials in order to make the device more efficient and less costly to manufacture. The results of one bioequivalence study comparing the two devices showed that multiple TI doses are additive, suggesting bioequivalence, albeit with larger confidence intervals [11].

TI technology has been shown to deliver insulin to the bloodstream with a PK/PD profile that closely mimics the physiologic postprandial endogenous insulin responses [12]. TI has a rapid systemic insulin absorption that achieves an insulin peak in plasma within 15 min, followed by a steep decline and a subsequent, more gradual return to baseline levels within 2 – 3 h [13]. The shorter duration of action and faster time-action profile distinguish this product from all others insulin forms [14-16].

Many clinical data (**Table 1**) have demonstrated that TI is non-inferior to diabetes medications now on the market and is able to offer comparable glycemic control with the advantage of less weight gain, reduced risk of hypoglycemia and reduced postprandial glucose (PPG) excursion. Studies of the variability of insulin levels achieved with TI revealed that MannKind's drug–device combination yielded more reproducible results than those achieved with SC administrations, with an inter-individual variability of 19.6 versus 50.1% [17-19], and relative bioavailability ranging between 20 and 25% [20] compared with subcutaneously administered human insulin.

Regarding potential long-term toxicity concerns for inhaled insulin, current studies with both TI [16,21,22] and Exu [23] indicate that inhaled treatment was well tolerated and no serious safety concerns emerged during the treatment, even after 4 years of inhaled TI therapy [22]. Early after therapy initiation (first 3 months) detected small changes in lung function, which remained non-progressive over 2 years of continuous TI therapy, and seems not to be clinically meaningful. In the same way, any alterations were observed in cardiac function and morphology in TI-treated diabetic patients. The most common adverse event associated with TI was transient non-productive cough, occurring within minutes of inhalation and characterized as mild to moderate [21]. According to MannKind, patients who might have impaired pulmonary function or are smokers, represent a small proportion of the total diabetes market and for that reason the company is not seeking approval of Afrezza in these cases [13]. However, some studies have already suggested that TI administration by these subjects do not alter insulin PK profile [24].

In a market survey conducted in 2009 involving 101 endocrinologists and 102 primary care physicians, and the second-generation inhaler of TI, physicians expressed a preference for TI for approximately 25% of both type 1 and type 2 patients that would qualify for use [3]. In the near future, this intention rate will be proven to prescribe TI in regular clinical practice.

2.2 Exubera

Exu consists in a dry-powder formulation with regular insulin (approx. 60%) and stabilizers, primarily mannitol, packaged in 1 and 3 mg blisters that contain approximately 3 and 9 U, respectively. Exu inhaler, Inhance™, with a size of 20 × 4 cm, has a base into which the blister is placed and through a pulse of compressed air, the dry-powdered insulin formulation is deagglomerated into an aerosol cloud, which is visible in the clear chamber of the device and ensures patient feedback via dose visualization while separates aerosol cloud generation from the inspiratory effort.

Insulin treatment with Exu was found to have PK/PD properties comparable with injected RAI aspart [25], and a faster onset of action and metabolic response than RHI [26] showing a relative biodistribution (BD) of 18% in the first 60 min after inhalation [27].

Exu has proven that it can provide glycemic control comparable with a conventional insulin regimen in both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) patients presenting similar adverse event profiles except for a higher incidence of cough in patients receiving Exu [23,28-31]. Less weight gain was also observed after 6 months using regimens including Exu compared with SC insulin-only regimens in T1DM and T2DM patients [32]. More long-term data have confirmed that these differences are sustained over 2 years [31,33]. Even after withdrawal of Exu, some studies have proven that inhaled insulin can improve significantly the glycemic control in difficult-to-treat patients due to needle phobia, injection site problem [34] or severe resistance to SC insulin [35], being well tolerated and greatly appreciated by these patients. More recently, a 24-week trial has shown that once- or twice-weekly insulin titration regimen of premeal Exu added to oral antidiabetic (OAD) therapy can safely achieve values of glycated hemoglobin (HbA_{1c}) equal or less than 7% in most T2DM patients inadequately controlled by only taking OAD therapy [36].

Lung function changes observed during discontinuation and re-administration of Exu therapy were likewise TI consistent with a reversible, non-progressive and non-structural pathological effect on lung function in adults with T2DM, as well was not associated with an augmented insulin antibody response [35].

Exu was tested under a wide range of typical use conditions and potential misuse scenarios [37] but premarket studies had excluded smoker patients and patients with comorbid illnesses such as epilepsy, asthma and chronic obstructive pulmonary

Table 1. Recent clinical studies related to the Afrezza® development.

Subjects/Treatment	Study design	Findings highlighted	Other remarks	Ref./Year
Comparative efficacy and safety studies				
n = 334/TI + glargine and n = 343/biaspart insulin (T2DM patients)	Randomized, open-label, parallel-group study (52 weeks)	Mealtime regimen with TI was found to be non-inferior to a regimen of twice-daily biaspart insulin. Fewer hypoglycemic episodes, both mild to moderate ($p < 0.001$) and severe ($p = 0.066$), as lower weight gain ($p = 0.002$) occurred in TI + glargine group	TI was well tolerated but was associated with increased frequency of cough, and an increased risk of infections, especially upper respiratory tract infections	[16]/2010
n = 301/TI + LAI and n = 288/RAI + LAI (T1DM patients)	Randomized, open-label, multicenter study (52 weeks)	TI group resulted in comparable HbA_{1c} reductions, but more favorable 1-h PPG and FPG, significantly less weight gain and risk of hypoglycemia	There were no differences in pulmonary safety monitoring results	[128]/2009
n = 126 (T2DM patients suboptimally controlled with OAD agents)	Double-blind, placebo-controlled, randomized, multicenter, parallel-group study (12 weeks)	TI was well tolerated and demonstrated significant improvement in glycemic control with clinically meaningful reductions in HbA_{1c} levels and PPG concentrations	Incidences of hypoglycemia, hyperglycemia, cough and other adverse events were low in both groups. Body weight was unchanged in both groups	[129]/2008
Dose response profile in PPG and metabolic control				
n = 227/14, 28, 42 and 56 U TI doses or placebo plus insulin glargine (T2DM patients with suboptimal glycemic control)	Prospective, multicenter, double-blind, placebo-controlled study (11 weeks)	TI plus basal insulin glargine is well tolerated and results in dose-dependent reductions in PPG and HbA_{1c} levels	There were no clinically relevant changes in pulmonary function tests, body weight or other medical analysis. Rates of cough were low and similar among all groups	[130]/2008
Pulmonary function over 2 years in diabetic patients treated with prandial TI				
n = 730/TI and n = 824/usual care (T1DM and T2DM adults)	2-year randomized, open-label study	Observed changes in lung function with TI were small, occurred early after therapy initiation, remained non-progressive over 2 years and were unlikely to be clinically meaningful	TI was well tolerated; no serious safety concerns emerged. The most common respiratory event associated with TI was mild, transient cough, occurring within minutes of inhalation	[21]/2012

DL_{CO}: Carbon monoxide diffusing capacity; FEV_1: Forced expiratory volume in 1 s; FPG: Fasting plasma glucose; HbA_{1c}: Glycated hemoglobin; ITQ: Insulin Treatment Questionnaire; LAI: Long-acting insulin analog; OAD: Oral antidiabetic; PPG: Postprandial plasma glucose; RAI: Rapid-acting insulin analog; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; TI: Technosphere® insulin.

Table 1. Recent clinical studies related to the Afrezza® development (continued).

Subjects/Treatment	Study design	Findings highlighted	Other remarks	Ref./Year
Assessment of the pulmonary function after cessation of TI				
n = 315 (121 T1DM, 194 T2DM)/ TI and n = 334/usual care	Follow-up trial after 1 and 3 months of the parent trial	The small, non-progressive dierences from baseline FEV_1 and DL_{CO} disappeared in T1DM and T2DM patients indicating that changes in pulmonary function are reversible when treatment has ended	There was no meaningful difference in FEV_1 between T1DM and T2DM groups	[131]/2009
Assessment of the perception of insulin therapy and treatment satisfaction				
n = 618/TI + glargine and n = 316/biaspart insulin	Multicenter study of adults with type 2 diabetes	Improvements in perceptions of insulin therapy, treatment satisfaction and treatment preference did not differ between groups	Perceptions of insulin therapy, treatment satisfaction and treatment preference improved in both arms (all $p < 0.001$)	[132]/2011
n = 58/active TI arm and n = 61/ placebo arm	Randomized controlled trial with insulin-naïve subjects	Perceptions of insulin therapy improved significantly during the trial in the active medication arm	These results came from the measures of health-related quality of life (the SF-36) and treatment satisfaction (the ITQ) before starting insulin treatment and approximately 12 weeks later	[133]/2010

DL_{CO}: Carbon monoxide diffusing capacity; FEV_1: Forced expiratory volume in 1 s; FPG: Fasting plasma glucose; HbA_{1c}: Glycated hemoglobin; ITQ: Insulin Treatment Questionnaire; LAI: Long-acting insulin analog; OAD: Oral antidiabetic; PPG: Postprandial plasma glucose; RAI: Rapid-acting insulin analog; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; TI: Technosphere® insulin.

disease, which led to contraindication in patients with these pulmonary conditions.

Treatment satisfaction with Exu was demonstrated in several large studies with T1DM and T2DM patients [29,38] before being marketed. These studies reported that the mean overall satisfaction and the quality-of-life scores improved significantly for the inhaled group and worsened slightly for the SC group. However, the related social comfort wasn't satisfactorily improved [39]. Those questionnaires slightly predicted some of the several limitations (**Table 2**) that could not be well accepted from patient's and physician's point of view.

2.3 AERx iDMS

AERx iDMS is another developed product which allows the delivery of insulin via inhalation but in a liquid form. It consists of a disposable prefilled sterile liquid insulin (AERx strip) with an integral nozzle, from which drug is aerosolized in particles of 2 – 3 µm via AERx Essence® device that delivers a pre-set dose in a single breath at correct rate and depth of breathing. The device has capabilities of precise dose adjustments and data capture for dosing and compliance monitoring [40]. This system was very well accepted in a 12-week multicenter open trial that revealed an excellent compliance with its dosing, timing and inhalation technique by T2DM patients [41]. Compared with RHI, the PK/PD profile of inhaled insulin with this system is similar, but with a more rapid onset of action [42] and with a relative bioavailability of approx. 13% [43]. In individuals with diabetes, preprandial inhaled insulin via AERx iDMS is as effective as preprandial SC insulin injection in achieving glycemic control (HbA_{1c} reduction) with similar tolerability in both T1DM [44] and T2DM [45] following intensive therapy (SC fast-acting insulin or inhaled insulin before meals, and SC intermediate-acting insulin at bedtime). However, the latest results suggest that inhaled insulin using the AERx iDMS requires further optimization to reduce nocturnal hypoglycemia and improve device dose delivery [44].

2.4 Advanced inhalation research technology

Advanced inhalation research or AIR technology is characterized by low-density particles with a diameter between 5 and 30 µm designed to allow an efficient drug delivery to the deep lung using a breath-activated device [46]. AIR system can be used in an intuitive manner and has proven that it requires minimal patient education, which may improve overall medication compliance [47]. Phase I clinical trials have demonstrated that AIR insulin is rapidly absorbed with prolonged insulin exposure and action compared with insulin lispro [48]. It also exhibited dose strength interchangeability and dose proportionality after single-dose administration in healthy subjects [49]. In T2DM patients inadequately controlled by one or more OAD medications, treatment with AIR insulin resulted in similar improvement in glycemic control compared with insulin lispro [50]. On one hand, the last Phase III clinical trial evidenced that AIR insulin was less efficacious in lowering HbA_{1c} than SC insulin in T1DM patients receiving intensive insulin therapy [51]. By contrast, AIR treatment results in more frequent cough and greater decrease in diffusing capacity of the lung for carbon monoxide. Moreover, in a 9-month study where patients with diabetes not optimally controlled by two or more OAD medications were involved in a shared decision-making approach to insulin treatment choices showed that the opportunity to choose AIR insulin did not affect overall use of insulin at end point or HbA_{1c} outcomes [52]. On the other hand, in a 12-week trial 80% of T1DM patients preferred AIR insulin to injected insulin, and a greater treatment satisfaction, comfort and confidence were significantly noted [53].

2.5 Aerodose

Aerodose insulin inhaler, a product developed by Aerogen, is based on its proprietary OnQ® aerosol generator technology, a breath-activated system able to deliver insulin to the deep lung using a liquid insulin formulation that is aerosolized in small droplets of approx. 4 µm of mean size [54]. The administration of higher insulin doses showed to be proportional with increasing slow deep inhalations, presenting relative bioavailability around 18 – 22% with no significant difference among doses [55]. Inhaled insulin by Aerodose inhaler also showed earlier time to maximum serum insulin (T_{max} 76 ± 51 vs 193 ± 66 min) and physiologic effect ($TGIR_{max}$ 170 ± 53 vs. 244 ± 75 min) compared with SC insulin ($p < 0.001$) with comparable dosing reproducibility [56].

A new partnership created in January 2011 between Aerogen and Dance Pharmaceuticals, a biotech company expert in the inhalation of peptides, is motivated in the development of a next-generation inhaled insulin product based on OnQ aerosol generator technology, and is likely to be even more stimulated when Afrezza shows its market performance.

2.6 Protein matrix microspheres

ProMaxx® (from Protein Matrix microspheres) is a drug delivery technology that allows the production of protein-loaded microspheres through a simple but robust process that preserves the protein structure and gives a narrow size distribution proper for pulmonary drug delivery [57]. The process involves a temperature-controlled precipitation of an aqueous insulin solution in the presence of polyethylene glycol (PEG) that after freeze-dried results in dry-powder microspheres of 1 – 3 µm of size composed of more than 95% of recombinant insulin [58] and aerodynamic stability over 10 months [59]. In a Phase I study [60], ProMaxx-inhaled insulin showed a faster onset of action compared with SC insulin with similar duration of action as total metabolic effect, and a bioavailability of 12% similar to other inhaled insulin formulations [61]. Optimization of the inhaler used to deliver ProMaxx insulin as microspheres with a different diameter, which can be easily formulated by the ProMaxx technology, may allow further

Table 2. Exubera® failure causes.

Safety and efficacy	• Treatment safety and efficacy comparable with other insulin regimens except for the higher incidence of cough with inhaled insulin
Long-term safety	• Uncertainty related to long-term safety of a new product. • Clinical trials showed reversible reduction of pulmonary functions and increase of insulin antibodies, which was considered not clinically meaningful but brought a general concern
Inconvenience	• Inhaler device aspect: bulky, not discrete. • Time-consuming management (blisters insertion, air pump activation, inhalation). • Regular check of lung function. • Previous adequate training effort. • Treatments with basal insulin still require routine injections
Cost	• The cost of Exu is much higher than injected insulins. • Limited insurance coverage for both therapy and pulmonary function testing
Dosing	• Exu doses differed from conventional units, thus conversions can be a source of confusion and error. • Inability to deliver precise insulin doses (the smallest blister pack available contains the equivalent of 3 U of regular insulin)

Adapted from [134].

improvements in the delivery and pharmacological properties of this inhaled insulin.

3. Nasal delivery systems

Intranasal delivery avoids many of the problems associated with other delivery routes, such as gastrointestinal degradation and first-pass metabolism associated with oral delivery, the pain and inconvenience associated with SC delivery, potential effects on lung function associated with pulmonary delivery and the need for specialized applicators and patch rotation associated with transdermal delivery. However, intranasal delivery also experiments some challenges such as the limited permeability of large molecule therapeutics through the mucosa, the need for some drugs to be formulated at high concentrations due to the acceptable dose volume limits between 100 and 200 µl per nostril, and rapid mucociliary clearance.

During last years, two different technologies reached the Phase II of clinical development for nasal insulin delivery, both representing ultra-rapid-acting intranasal insulin formulations. One is Nasulin™, a nasal spray formulation of insulin developed by CPEX Pharmaceuticals, Inc. Nasulin contains RHI dissolved in sterile water in combination with polysorbate 20, sorbitan monolaurate, cottonseed oil and its key and proprietary excipient cyclopentadecalactone, CPE-215, as transmembrane absorption enhancer [62]. Initial and small studies in healthy volunteers and T1DM subjects show that Nasulin has a relative bioavailability of approximately 15 – 20% compared with SC insulin and is generally well tolerated. The absorption of insulin in this formulation results in a rapid onset of action (10 – 20 min) peaked at 30 – 50 min and with an appropriate duration of action (1.5 – 2 h) [63,64]. In a second study, PK and PD parameters showed proportional and linear dose responses with the two concentrations of Nasulin applied, making multiple doses available for clinical development and greater titration flexibility [65]. Nevertheless, the intrasubject variability observed with Nasulin administration was always high (~ 40%) [62,66]. In March 2010, the company reported the preliminary results of the Phase IIa study designed to assess the glycemic control in T2DM patients (n = 94). After 6-week treatment based on basal glargine insulin injection in the morning and two (50 IU) or four (100 IU) sprays of Nasulin at 1%, or placebo, three times daily prior to meals did not produce differences statistically significant between groups. Controversially, subjects in the placebo group spent less time reaching euglycemia compared with subjects in the Nasulin group [67]. After analysis of these preliminary outcomes, CPEX in 2010 decided to stop further Nasulin development activities, seeking to out-license its program [68,69].

Nastech Pharmaceutical is another company that developed a rapid-acting intranasal insulin formulation, which is currently property of Marina Biotech. The results from a Phase II clinical trial with 29 T2DM patients revealed that the novel ultra-rapid-acting intranasal insulin with a bioavailability of 17 – 28% was both superior to usual therapy (OAD medicines and/or basal insulin) and non-inferior to the injectable RAI aspart in maintaining glucose control following a meal. The time to reach maximum concentration for intranasal insulin was faster (30 min) than for insulin aspart (90 min), which further demonstrates the ultra-rapid-acting nature of the product. Other aspects highlighted by the company were related to the safety advantage of lower rates of post-meal hypoglycemia with the new formulation, and the convenience of a nasal spray, which does not require refrigeration and is easy to use [70].

Currently, no further development is being addressed by the companies of these products until future new partnership investments appear.

Due to intranasal delivery limitations, it is typically necessary to include these formulations in excipients capable of

enhancing mucosa permeation, such as bile salts and derivatives, surfactants, fatty acids and derivatives, and various bioadhesive excipients (e.g., chitosan), because the bioavailability of intranasally administered insulin without absorption-enhancing agents is only 1 – 2%. Subsequent local irritation and other adverse effects usually associated with nasal administration make this option a limited practical application for long-term therapies. The most usually described adverse effects and symptoms are sneezing, running nose, watering eyes and stinging or burning sensation. Other more transient and less frequently reported symptoms include tickling or tingling, irritation, unpleasant taste or smell, headache, cough and nasal congestion [62,65]. Longer-term studies will be needed to see if chronic administration to the nasal mucosa would lead to chronic inflammation or other adverse local events.

Since the latest evidences that insulin administered intranasally improves memory in healthy humans and cognitive function in Alzheimer's disease patients [71,72], this novel route of administration of insulin can experiment an exceptional overturn in future clinical developments. Interestingly, a recent double-blind, placebo-controlled trial showed that an intranasal insulin treatment (20 IU insulin/day) preserved not only general cognition but also reduced the loss of metabolic integrity of the brain in adults with amnestic mild cognitive impairment (n = 64) and with mild-to-moderate Alzheimer's disease (n = 40) [73].

4. Oral delivery system

Oral route has largely been studied as a novel alternative to SC insulin injections. This route can be considered the most patient-friendly way of insulin administration and hence it has high potential to facilitate early insulin therapy initiation and offer a better patient compliance. Furthermore, in this way insulin is absorbed at gastrointestinal tract (GIT), easily reaches the portal vein and is directly transported to the liver, mimicking the pathway of insulin endogenous, allowing a better glucose swing control and decreasing the peripheral hyperinsulinemia [74].

However, insulin is a protein with high molecular weight, poor lipophilicity, low epithelial permeation and last, is able to suffered enzymatic degradation along GIT. Thus, insulin has low oral bioavailability, but some efforts are being developed to overcome this limitation by means of absorption enhancers, enzyme inhibitors, chemical modifications or new insulin carriers [75].

4.1 Capsulin

Diabetology developed a new technology that enables a protein and peptide delivery, Axcess™ Delivery System, an enteric-coated capsule for gastric protection, containing drug and a mixture of absorption enhancers and solubilizers that allow absorption of the drug in the small intestine. Both components are registered pharmacopoeial or generally recognized as safe (GRAS) excipients with a long story of pharmaceutical use. Thus, Capsulin™ OAD uses the Axcess Technology as a novel oral insulin delivery system for T2DM.

Phase I clinic trial results showed that system was tolerable by the volunteers and the blood glucose reduction and the suppression of peptide-C indicate that this system has a good bioavailability and it is biologically active [76]. Moreover, Phase II clinic trials have been completed. Some PK and PD results in T2DM patients were reported when SC human insulin and two different oral doses of Capsulin (150 U and 300 U) was compared by the measurement of glucose infusion rate (GIR) and plasma insulin concentration (**Table 3**) [76,77].

Both doses of Capsulin had a GIR significantly elevated after 6-h clamp period, indicating its effect exceed 6 h; however, SC human insulin's GIR was higher than both Capsulin doses. In turn, SC human insulin had a significant higher plasma insulin concentration than oral insulin system studied, but no difference was observed between 150 and 300 U Capsulin. Therefore, administration of Capsulin oral insulin to patients with T2DM demonstrated a significant hypoglycemic action throughout a 6-h isoglycemic clamp procedure, and with only a small increase in peripheral circulating plasma insulin.

A repeat-dosing study was also performed where self-monitored blood glucose was achieved in 10-day period with 150 U at a dose of Capsulin, twice a day. This study showed that system was tolerable and safe for the patients, and at the post-study there were improvements in HbA_{1c} and triglycerides levels and weight [77].

In turn, it has been considered as an alternative to inhaled and injected insulin systems for T1DM or late stage of T2DM, being in Phase II stage of development. The main Phase IIa conclusion study showed that not only the system is safe and well tolerable, but can also produce a consistent increase of insulin blood level in 30 – 120 min after oral administration. It also showed that insulin level is maintained during an extended time period and provide both blood glucose control in a dose-dependent manner and an increased insulin level. The study also predicted that oral insulin delivery system is able to restore the hepatic control of insulin regulation [76].

4.2 ORMD-0801

Oramed Pharmaceuticals developed a new oral insulin delivery, known as ORMD-0801, an enteric-coated capsule that protects insulin against GIT degradation by incorporating adjuvants which promote the transport across wall intestinal and insulin uptake [78,79].

The safety, PK and PD properties of this system, when administered in T1DM patients, were evaluated through a Phase IIa study. After oral administration of two capsules of ORMD-0801 (8 mg insulin each), a standard meal was served at 10, 45 and 90 min. Insulin and PPG levels were monitored over 6 h after oral administration [80]. This study concluded that this system is safe, biologically active when administered

Table 3. GIF and insulin concentration after 6-h SC human insulin and Capsulin™ administration; n = 16; p < 0.05 statistically significant.

		SC human insulin (12 U) A	Capsulin (150 U) B	p-value A vs B	SC human insulin (12 U) C	Capsulin (300 U) D	p-value C vs D
GIR	$AUC_{0-6\ h}$ (g/kg)	0.51 ± 0.15	0.31 ± 0.09	**0.036**	0.60 ± 0.26	0.30 ± 0.11	**0.017**
	C_{max} (mg)	2.14 ± 0.58	1.50 ± 0.43	**0.017**	2.49 ± 1.25	1.42 ± 0.44	**0.036**
	T_{max} (min)	283.8 ± 57.3	197.5 ± 93.3	0.080	245.0 ± 92.1	242.5 ± 83.3	0.866
Insulin concentration	$AUC_{0-6\ h}$ (pmol h/l)	910 ± 269.7	472.1 ± 244.7	**0.012**	950.2 ± 446.7	433.3 ± 218.9	**0.012**
	C_{max} (pmol/l)	197.9 ± 50.4	104.9 ± 48.3	**0.012**	206.1 ± 114.5	137.1 ± 111.6	0.123
	T_{max} (min)	126.3 ± 80.0	156.3 ± 60.0	0.182	208.8 ± 57.4	213.8 ± 74.1	0.944

Adapted from [77] with permission of John Wiley and Sons.
AUC: Area under the curve; GIR: Glucose infusion rate.

by preprandial oral route and it is cleared in 300 min. This study also showed that on insulin removal ORMD-0801 was able to control the glucose levels in fasting T1DM individuals [80].

The safety, tolerability and efficiency of oral insulin ORMD-0801 versus placebo was evaluated trough a Phase IIb in T2DM patients during 6 weeks. The patients received a capsule of ORMD-0801 or placebo at bedtime. The study showed significant reduction of insulin, C-peptide and fasting blood glucose (FBG); however, HbA_{1c} levels were higher in experimental group than placebo. It was concluded that ORMD-0801 can cause some hypoglycemic effect when administered at bedtime. Therefore, this first long-term study proved that this system can be safe, well tolerable with no adverse accumulative effects [81].

4.3 Eligen Technology

Eligen® Technology is a new system created by company Emisphere Technology, which uses a delivery agent to carry on molecules, like insulin, across the biological membranes, avoiding the acid and peptidase degradation. These carriers interact with protein drugs in a weak and non-covalent manner, changing their conformation and increasing their lipophilicity, allowing their absorption by passive transcellular transport [74,82].

In a study performed in 10 fasted healthy volunteers, oral insulin administration in combination with sodium *N*-8-(2-hydroxybenzoyl) aminocaprylate (SNAC) showed that insulin was rapidly absorbed into the bloodstream (C_{max} occurred within 25 min). In other clinical study in T2DM patients, one capsule containing 10 mg of insulin and 200 mg of SNAC was able to reduce PPG levels and increase systemic insulin level over 30 min after a standardized meal.

Despite the fact that it has been considered as an alternative for therapeutic protein delivery system, the formulation is responsible for causing nausea and there is no clinical efficacy study to date. Moreover, delivery system may be inconvenient for patients because the used amount is quite higher than the therapeutic proteins [83].

The 90-day Phase II study of high doses of oral insulin using Eligen Technology in T2DM showed an HbA_{1c} reduction [82]. In another Phase II clinic trial by Emisphere Tecnnology, oral insulin combined with metformin did not present higher glycemic control as compared with metformin-only treatment (group control) [84].

In 2010, the company made an agreement to develop and commercialize oral insulin analogs patented by Novo Nordisk, using Eligen Technology [85].

4.4 Oral insulin delivery matrix

BOWS Pharmaceuticals established a system based on RHI in dextran matrix capsule, which avoids and protects the insulin against proteolytic degradation. It also allows a better insulin target of local absorption when dextran degraded by endogenous enzymes presents in small intestine mucosa [78,86]. A Phase I/II study using a peroral capsule containing human insulin in a dextran matrix (ORA2) was performed with the goal of assessing the safety, tolerance and PD profile in T2DM patients. However, this study was suspended in 2010 [87].

Merrion Pharmaceuticals developed Gipet® Technology that uses drug absorption enhancers through matrices constituted by medium-chain fatty acids (recognized as a GRAS excipients) [74]. The insulin and the other ingredients are mixed through a physical mix and are formulated as solid dosage form, a tablet, which just allow the insulin release in duodenum, allowing the GIT transport and insulin absorption [74,88].

In turn, Merrion Pharmaceuticals established a partnership with Novo Nordisk which pretend to use Merrion's proprietary Gipet with an insulin analog patented by Novo Nordisk. In 2012, Novo Nordisk announced the ending of a single-dose Phase I clinic trial of oral insulin 106 in a Gipet I Gastro-resistance tablet (NN1952) in volunteers with T1DM and T2DM [88,89]. It was concluded that single doses of oral insulin were well tolerated, and when administered in fasting states has a rapid absorption and onset of action. However, PK and PD parameters had a large inter-individual variability when compared with SC insulin. The study also showed that absorption changed by the presence of food (80 – 90% the AUC (area under the curve) of formulations decreased). Furthermore, the clinical development project was discontinued by Novo Nordisk [89].

4.5 Hepatic-directed vesicle insulin

Diasome Pharmaceuticals has developed a novel insulin delivery system called hepatic-directed vesicle insulin (HDV-I). Insulin is bonded to a hepatic-directed vesicle (HDV) and, at the phospholipid bilayer of liposome there is a hepatocyte-targeting molecule (HTM), which allows a better targeting of insulin in liver cells, mimicking the physiological conditions. That system is also able to avoid the insulin degradation caused by proteolytic enzymes present in GIT [74,90,91].

Phase II clinic trials showed an improvement in blood glucose control during an oral tolerance test in diabetic patients. Another study was conducted to assess the dose-response of PPG through escalating daily doses of oral HDV-I in T2DM patients, along with OAD therapy. The study showed a statistically significant reduction of PPG for all doses of oral HDV-I when compared with placebo [92].

Another study compared the glucodynamic profile of oral HDV-I with a SC RHI during 14 days in T1DM patients, by the evaluation of 7-point blood glucose tested in 4 days, FBG and PPG levels. No statistical differences were achieved in 7-point blood glucose, FBG and PPG parameters between oral HDV-I and SC insulin. But a significant decrease in overall mean daily value between two groups occurred, likely due to small size of the sample. So, when compared with SC insulin, oral HDV-I treatment significantly reduced the mean daily 7-point blood glucose in T1DM [93].

4.6 Biocon's insulin analogs

Another short-acting insulin analog developed by Biocon is IN-105, which consists of an insulin molecule modified with a short chain of PEG [78,94]. In a study, the PK parameters of different doses of IN-105 (10, 15, 20 and 30 mg) were evaluated and compared with placebo in T2DM. The C_{max} of insulin reached at 20 min post-administration and insulin levels returned to baseline 80 min after administration. In turn, blood glucose reached the minimum concentration at around 40 min. It was concluded that reduction of PPG occurred in a dose-dependent manner. Furthermore, in previous clinical studies, it was noted that the onset of action of insulin occurred within 10 min after injection and the duration of effect was over 1.5 to 2 h [95]. In 2009, Biocon announced the beginning of Phase III clinic trials in T2DM patients [94].

4.7 Vitamin B_{12} to deliver oral insulin

In order to improve the intestinal absorption of proteins, Access Technology developed a novel oral drug delivery technology named CobOral™. This technology is based on vitamin B_{12} properties and its natural transport system, which allows co-absorption of the drug during vitamin uptake [96].

To enhance the drug absorption, Cobalamin™ or vitamin B_{12} analog and drug can be linked at polymers, or drug can be encapsulated in a Cobalamin-coated nanoparticles. As Cobalamin binds to intrinsic factor, it is in turn linked to a receptor, where drug–Cobalamin conjugate is absorbed by mediated endocytotic processes that allow the permeation through intestinal wall. An *in vivo* study of Cobalamin-coated insulin-loaded nanoparticles had a pharmacological response (blood glucose decrease), 80% equivalent or greater than SC insulin. The system also showed a slower onset and a longer duration of action when compared with SC route [96]. The beginning of Phase I clinical trial has been announced but so far there are no results available [97].

5. Buccal insulin delivery system

Buccal route has an easy access to systemic circulation without passing by first-pass liver and GIT degradation, increasing the bioavailability of drugs. Due to its permeability and large surface, buccal mucosa allows the passage of large molecules [98,99].

Generex Biotechnology Corp. developed a new buccal insulin delivery system called Oral-Lyn™, which uses a metered dose spray device, RapidMist™, where drug is put into a solution with an appropriate combination of enhancer absorptions and GRAS excipients [83,98]. This system is able to produce reproducible doses, with an easy handling in patient-friendly manner. Each dose delivers 10 U of insulin but only 10% is absorbed, whereby the system is suitable for prandial insulin delivery, immediately prior to breakfast [100].

Buccal insulin delivery system is safe and effective; therefore, it can be used for both T1DM and T2DM patients and is considered as an alternative to SC injections [98]. This system has been marketed at India, with the name brand Oral Recosulin™, however Phase III clinical trials have been performed around the world [83,101].

In turn, Monosol Rx has developed an alternative technology to pills, gels and injections, PharmFilm® Drug Delivery Technology. This system uses a film, with a small size, thickness and shape, allowing a uniform drug delivery through buccal, sublingual, enteral or vaginal routes. PharmFilm Technology is a versatile easy-to-use technology, it dissolves quickly without taste, so that it can be used in different patients, like pediatric, psychiatric and geriatric populations [102]. Monosol Rx in collaboration with Midatech Group has developed a new nanoformulated transbuccal insulin film, Midaform Insulin PharmFilm, which employs the rapid-soluble PharmFilm Technology for delivering insulin-passivated glyconanoparticles developed by Midatech Ltd. The Phase I clinical trial results announced in 2012 showed that this system is safe, has a rapid onset of action and allows to mimic the monomeric insulin secreted by pancreas [102,103].

6. Transdermal delivery systems

The skin is the largest organ of human body and has been used as a route for macromolecules delivery, like insulin. This non-invasive route offers a good way for absorption of insulin until bloodstream, enabling a good control of diabetes diseases and avoiding the pain of using needles [99]. It also avoids the gastrointestinal degradations and the metabolism

of liver. However, the skin is a good and functional barrier of human body, with low permeability that prevents the passage of drugs, especially the hydrophilic macromolecules, such as insulin [99,104].

To overcome the low permeability of insulin into the skin, some techniques have been developed that allow the transdermal insulin delivery system.

6.1 Microneedles

A novel approach to increase the transdermal insulin delivery is based on the use of microneedles, needles that are fabricated in a microscale, generally with a mean diameter of 1 micron and a length range over 1 – 100 microns [105].

These systems are produced in a reproducible manner with a high precision and moderate cost, through the use of several materials, like silicon, metals, polymers, silicon dioxide, and glass [105,106]. With their microscale size, they can pierce the skin opening microchannels larger than macromolecules size, allowing the transport and the permeability of drugs, like insulin. Besides micropathways created by microneedles, they are able to penetrate only into the stratum corneum due to their microsize (with a thickness of 10 – 15 μm), thereby not reaching the nerve endings, avoiding the pain and fear associated with needles [99,105,106].

Microneedles can be combined with a transdermal patch, in other words, a patch system which has an array of microscopic needles, providing a minimal invasive transport of insulin until bloodstream [107]. This discrete system has several advantages of a traditional transdermal patch technology, such as continuous and controlled release, easy to use, minimum involvement of healthcare professionals support and painless [108].

Passport™ System is a transdermal patch technology created by Altea Therapeutics Corp., which allows efficient and fast delivery therapeutic proteins. Due to its capacity of basal insulin delivery, it has been clinically studied in T1DM patients, and Phase I trials have already shown a sustained insulin delivery over a period of 12 h [109]. In 2007, a Phase II clinical trial was completed, and results revealed that insulin level achieved with transdermal system was equivalent to one SC long-acting insulin (LAI) analog marketed [87,109,110].

Altea Therapeutics's system has an applicator and a PassPort Patch which contains a drug reservoir and a small screen, also called porator that contains metallic filaments [109,111]. The applicator releases an electric charge to the porator, where electrical energy is converted in thermal energy at the patch's filaments. That way, hundreds of microchannels in the keratinized layer of skin are formed, where the insulin can pass until the blood circulation. No pain is felt because the amount of thermal energy used does not penetrate so deeply, not attaining the sensitive nerve endings in the dermis [109].

Valeritas, Inc. has already its transdermal patch in the market and it is known as V-Go™. It is a fully disposable insulin delivery device that can be used with Novo Nordisk's NovoLog® and Eli Lilly's Humalog® [112]. It employs the h-Patch™ technology, a disposable device which allows a controlled drug delivery through the combination of transdermal patch and conventional ambulatory pumps advantages [112,113]. The system is able to deliver a set basal dose of insulin (20, 30 or 40 U/24 h) and on-demand bolus insulin at mealtimes can be added at the same time (2 U for each administration, allowing 36 U bolus RAI during 24 h) [112,113]. V-Go is a simple and discrete device which can be applied in several parts of human body. It has a small size, low weight, an easy application (just by removing the adhesive strip can be applied directly into skin) and doesn't need needles, syringes, battery or electronic sources for insulin delivery. However, prior to use, a new device is required to fill V-Go insulin reservoir [113]. After skin application, patients need to press a button for basal insulin administration. A second button is required to be pressed for mealtime bolus delivery [113,114]. The h-Patch can be used just during 24 h, and after its removal, the microneedles retract being blocked, which means that the device is useless for re-use. FDA approved its marketing for T2DM treatment and in 2011 it received approval for being marketed in Europe [112,114].

6.2 Iontophoresis/sonophoresis

Iontophoresis is a technique that uses small electric currents for enhancing permeability of drugs into skin. This method allows a continuous, pulsatile, controlled and non-invasive drug delivery using processes of electromigration and electro-osmosis [99].

A study reported iontophoresis can have a synergic effect for insulin delivery with microneedle-induced microchannels. This study in rats showed that iontophoresis drives the insulin-loaded nanovesicles into the deep layers of the skin over microchannels, where it can be released, followed by the absorption [115].

Another approach for insulin delivery is sonophoresis, which uses ultrasound waves for delivering therapeutic molecules across the skin by the use of high-frequency sonophoresis (HFS), frequencies upper than 0.7 MHz [116] or low-frequency sonophoresis (LFS), frequencies between 20 and 100 kHz [116,117]. Several studies have shown that HFS is able to enhance the permeability of molecules with low molecular weight, as non-steroidal anti-inflammatory drugs (NSAIDs) or local corticosteroids delivery [116], but no good outcome was found for large hydrophilic molecules until Mitragotri *et al.* published in 1995 the first *in vivo* study which showed that application of ultrasounds at 20 kHZ helped in delivery of insulin at therapeutic levels through hairless rat skin [118]. Thus, LFS was found to be much more effective for the delivery of hydrophilic macromolecular drugs such as insulin [116-118].

The sonophoresis mechanism is still not fully achieved, but it is believed that enhancement of skin permeability is due to cavitation effect [116]. This method comprises growing and oscillation of air pocket present on keratinocytes and which is responsible for lipid bilayer disorganization of stratum

corneum [118]. As the cavitation effect increases with the decrease of ultrasounds frequency [117,119], the use of LFS provides bilayer disorganization enough to allow the permeation of large molecules through the skin [118].

In order to have an appropriate sonophoresis efficacy, several parameters must be achieved, such as ultrasound energy dose, frequency, intensity, pulse length and distance of transducer from the skin [120]. However, sonophoresis can be combined with several strategies as chemical enhancers, iontophoresis or electroporation, for having a synergic effect and consequently a better skin permeability [121,122].

Transdermal Specialties, Inc. has developed a new transdermal system called U-Strip™ patch, a needle-free system that uses alternating ultrasonic waveforms to increase the pore diameter of skin, so that drugs, drugs like insulin can penetrate the skin and reach the blood [123]. The system has an insulin patch, where insulin is stored and transducer coupler is snapped. It also has a battery, an ultrasonic applicator, which is responsible for generating ultrasonic transmissions and a keypad to program drug dosage and frequency [124]. This disposable device can be used on the arm or abdomen and it is available in four different doses (25, 50, 100 and 150 U), allowing a bolus insulin delivery and a basal insulin delivery enough for 2 days [123,125]. This system is able to detect insulin level on the patch, making a sound alarm when battery is low, low insulin level on the patch or when the system falls off. The system is a closed-loop delivery which contains a glucose sensor, so that glucose readings can be registered. These data and the insulin amount that remains on the patch can be saved during 60 days. This device has also the ability to send a dose report directly to physician via internet for monitoring and provide updates to patients about insulin dose and glucose level information along the day [123-125].

Transdermal Specialties is currently involved in U-Strip clinic trials development. Just two clinical trials involving over 500 diabetic patients are required for FDA approval: a study to assess if blood glucose level of insulin patch can reach at the same time as pump, but with less amount of insulin. This trial also pretends to evaluate if that system is more effective in glucose reduction at morning for those patients who wake with high blood glucose levels; and a second one which will be conducted at patient's home to evaluate HbA_{1c} levels for over 4 months [125].

6.3 Transferosomes

Transferosome is a trade name registered by IDEA AG, which means 'carrying body'. It is an elastic, flexible and deformable vesicle which allows to squeeze itself in order to pass through the pores of the skin, even those pores with less size than the own vesicle. These vesicles are made using a phospholipid component, like phosphatidylcholine, to assemble the lipid bilayer and a mixture of surfactants, which in appropriate rate, is able to control the flexibility of vesicles and consequently their stability [126,127].

Due to their flexibility properties, these vesicles are able to penetrate into the skin through a transcellular route. When applied at skin, it follows the natural gradient of water across the epidermis to secure its adequate hydration, thus the transepidermal hydration is the driving force of transferosomes [126,127].

Transfersulin® consists of encapsulation of insulin into transferosome which is a good approach for overcoming the problems of SC insulin injection [127]. Unfortunately, no further information is available related to its status of development.

7. Conclusions

Diabetes management requires daily blood glucose control through a series of multifaceted actions but mainly by multiple daily insulin injections. Several non-invasive methods of insulin delivery have been created and largely developed using diverse strategies and following different routes of administration (**Table 4**) with the purpose of SC insulin replacement. These non-invasive methods promise a better diabetes care by giving improvements in blood glucose level control, and decreasing the weight gain, peripheral hyperinsulinemia, hypoglycemic events and late diabetic complications. Continuous studies covering all stages of the clinical development are being addressed, and most of them have evidenced good clinic results, but there are still many barriers to be overtaken. Inhaled insulin system, especially Afrezza, has proved its efficacy, safety and tolerability to SC insulin injections. In turn, some absorption problems must be overcome for nasal spray insulin being used. Oral route is one of the main strategies that have been developed. A solution has been achieved for poor insulin bioavailability and intestinal permeation; however, risk-benefit and long–term toxicity must be further studied. Apart from V-Go, there are others transdermal systems which use different strategies that need to be evaluated before being marketed.

Each route and delivery method has its own potential advantages and limitations, and maybe a universal suitability of a system cannot be possible. However, regardless of route administration, any non-invasive and alternative approach would revolutionize the current diabetes treatment and would be well accepted by the patients and healthcare professionals, particularly if it presents cost-effectiveness and user-friendly aspects.

DM is a disease that affects thousands of people around the world, and a single suitable pharmacological profile or public adherence is not enough to ensure the success of a novel insulin delivery system.

8. Expert opinion

Despite the main purpose of diabetes treatment, innovative technologies for non-invasive insulin delivery have been created under different multistrategic approaches and each one

Table 4. Non-invasive insulin delivery systems under clinical development or recent marketed.

Route of administration	Product name	Company (developer and partner)	Technology	Status	Ref.
Pulmonary	Afrezza®	MannKind Corp.	Technosphere® dry-powder and Dreamboat™ inhaler (breath-powered)	Market approval expected in 2012/2013. Running additional clinical trials	[3]
	Exubera®	Nektar Therapeutics and Pfizer (out since 2007)	Dry-powder and Inhance™ inhaler (breath-powered)	FDA and EC approval (January 2006) and withdrawn (October 2007), Phase IV (last update in January 2012)	[135]
	AERx® iDMS	Aradigm Corp. and Novo Nordisk (Novo Nordisk bought all the developing rights in 2004)	Liquid aerosol delivered by an electromechanical inhaler	Phase III (program cancelled, January 2008)	[136]
	AIR® insulin system	Alkermes and Eli Lilly	Dry-powder delivered by a disposable, breath-powered inhaler	Phase III (program cancelled, March 2008)	[137]
	Aerodose insulin inhaler	Aerogen, Inc. and Ypsomed (formerly Disetronic Medical	Liquid insulin aerosolized by a small, hand-held, breath-actuated inhaler	Phase II (placed on hold in 2003)	[138,139]
	-	Kos Pharmaceuticals (acquired by Abbott Laboratories in 2006)	Dry crystals delivered by a handheld, breath actuated inhaler driven by a propellant	Phase IIa (2004)	[140]
	ProMaxx®	Epic Therapeutics (acquired by Baxter Healthcare Corp.)	Dry powder delivery by a DPI	Phase I (2008)	[57]
	Qdose	Vectura and MicroDose Therapeutx (joint known as QDose Ltd.)	Dry powder delivered by a pocket-sized, multi-unit dose, piezo-electronic DPI	Phase I (2007)	[141,142]
	Spiros	Elan Pharmaceuticals (formerly Dura pharmaceuticals	Dry powder delivered by a handheld battery-powered multidose inhaler	Phase I (stopped in 2004)	[143]
	Alveair	Coremed/Fosun-Wanbang	Liquid insulin delivered by means a generic handheld device	Phase I (2004)	[144]
	Bio-Air	BioSante Pharmaceuticals	Coated dry particles based on calcium phosphate nanoparticulate delivery system	Phase I (2005)	[145]
Nasal	Nasulin™	CPEX Pharmaceuticals, Inc. (Bentley Pharmaceutical till 2008)	Spray formulation based on CPE-215, a transmembrane absorption enhancer	Phase IIa completed in 2010 (program for sale or out-license)	[69]
	-	Marina Biotech (formerly known as MDRNA, and Nastech Pharmaceutical)	Company's nasal spray formulation	Phase II incomplete (patent for sale since September 2011)	[70]

DPI: Dry-powder inhaler; HDV: Hepatic-directed vesicle; HTM: Hepatocyte-targeting molecule.

Table 4. Non-invasive insulin delivery systems under clinical development or recent marketed (continued).

Route of administration	Product name	Company (developer and partner)	Technology	Status	Ref.
Oral	Capsulin™	Diabetology	Axcess™ Technology	Phase II completed	[76]
	ORMD-0801	Oramed Pharmaceuticals	Enteric-coated capsule which contains adjuvants	Phase IIa in T1DM Phase IIb in T2DM	[143,146]
	-	Emisphere Technology	Eligen® Technology	Phase II (2006)	[82]
	ORA2	BOWS Pharmaceuticals	Insulin in dextran matrix capsule	Phase I/II suspended in 2010	[87]
	NN1952	Merrion Pharmaceuticals and Novo Nordisk	Gipet® Technology	Discontinued	[89]
	HDV-I	Diasome Pharmaceuticals	Insulin bonded to an HDV which contains HTM	Phase II	[90,91]
	IN-105	Biocon	Insulin bonded to PEG	Phase III	[78,94]
	-	Access Pharmaceuticals	CobOral Technology	Phase I	[96]
Buccal	Oral-Lyn™	Generex Biotechnology Corp.	RapidMist™ Technology	Phase III/marketed	[83,98]
	Midaform Insulin PharmFilm®,	Monosol Rx and Midatech Ltd.	Use of PharmFilm Technology for delivering insulin-passivated in glyconanoparticles	Phase I	[102,103]
Transdermal	Passport™ System	Altea Therapeutics	PassPort Patch combined with microneedles	Phase II in 2007	[87,109]
	V-Go™ Disposable Insulin Delivery Device	Valeritas, Inc.	h-Patch™ technology	Marketed	[112]
	U-Strip™	Trandermal Specialties	Use of ultrasonic waveforms to increase the pore diameter of skin	Phase III	[125]
	Transfersulin®	IDEA AG	Encapsulation of insulin into transferosome	Phase I in 2007	[126,127]

DPI: Dry-powder inhaler; HDV: Hepatic-directed vesicle; HTM: Hepatocyte-targeting molecule.

supply specific requirements inherent of respective administration routes. Each new method developed has its own limitations and potential advantages, and it is not possible to find a product for everyone due to product or individual contraindications. Nonetheless, a novel product will be as successful as higher its suitability among diabetic patients in general.

The clinical trial results have revealed a pronounced potential of these novel insulin delivery systems in DM treatment. Most of the studies evidenced that treatments with new delivery systems are comparable with standard of care insulin therapy, and promise a suitable coverage of prandial insulin requirements, which means they have the potential to replace the numerous short-acting insulin injections every day. However, in a general view, better comparator studies should be carefully designed. Comparison between different delivery systems of the same route of administration, comparison with more insulin analogs, with insulin delivered by pen devices or with continuous infusion systems are still to be performed.

Inhaled insulin therapy consistently demonstrated improvements in glycemic control, whether added to longer-acting SC insulin regimens in patients with T1DM and T2DM or used to supplement or replace OAD therapy in patients with T2DM. Inhaled insulin therapy with Afrezza showed not only to be efficacious, well tolerated and has long-term safety profile, but also to provide advantages of effective protection against postprandial hyperglycemia and less weight gain than conventional insulin therapies.

On the other hand, nasal insulin spray formulations face some absorption challenges and require further optimization. At least, if today the nasal insulin delivery doesn't sound a future non-invasive alternative in DM treatment, it is definitely a new promising approach to treat neurodegenerative disorders as some recent studies have shown.

As the most convenient administration route, oral insulin delivery systems have been considered a desirable and potential alternative to SC insulin injections. However, many barriers remain to be overcome. Oral insulin absorption has been proved, but the reproducibility of oral insulin absorption in a larger range of patients needs to be performed in order to evaluate the inter-individual variability. Phase III clinical trials results haven't been shown, and the changes of PK caused by the influence of others drugs or food still need to be evaluated. The risk-benefit and chronic toxicity administration must be assessed, as the extended use of enhancer absorption, which increases the intestinal permeability, could increase the absorption of other compounds in particularly toxins or even pathogens agents. The use of protease inhibitors has been studied as an approach for oral insulin delivery, needs to be carefully assessed as they might inhibit some useful enzymes for digestive process. Another barrier is the possible mitogenic activity caused by insulin which could cause mutagenic changes in intestinal mucosa, therefore long-term safety studies must be performed.

In the same way, transdermal route can offer a good approach to SC insulin, once it can provide a controlled and continuous drug delivery. Microneedles combined with patches have been studied for other disease treatment or vaccination with good results. Undoubtedly, V-Go Disposable Insulin Delivery Device could revolutionize the diabetes care. However, the system applicability to others insulin analogs, and long-term safety, should also be assessed. Due to bioengineering development, the combined use of microneedles with other active enhancement technologies, like iontophoresis and sonophoresis, can be a good strategy to overcome the several skin barriers. In turn, transferosomes are able to permeate stratum corneum, allowing insulin delivery due to their flexibility. Little information about their status of development was found, probably because these systems can suffer oxidation and natural phospholipids have poor purity.

At this time it is not possible to say that certain non-invasive insulin delivery system is better than traditional diabetes treatment. Nonetheless, a non-injectable alternative system of insulin delivery will be attractive to fight psychological insulin resistance in both patient- and physician-related barriers that contribute to the late initiation of insulin therapy and consequently further DM complications. In fact, the possibility to achieve a good glycemic control through any reduction of HbA_{1c} and additionally without being associated with weight gain and increased hypoglycemia will be welcomed, but it does not guarantee overall adherence. From the patient's point of view, it is comprehensible that a diabetic person, who has to follow several self-management tasks (including physical activity, healthy eating, taking medications correctly, glycemic self-monitoring, among others), just will perfectly comply with a new insulin product if it will have an easy handling and do not yield more complicated issues. The acceptance of a new alternative insulin treatment will also result from the inherent judgment and comparison between the current well known, safe and effective treatments, and respective cost-effectiveness rates. The price is always an unavoidable criterion. The companies of the new products will have to make an extra effort with a solid marketing strategy to show characteristics of its product when it hits the market. Obviously, manufacturing process should be as simple as possible and at the same time be able to produce at high scale to ensure all diabetic market supplying. In the near future, it will be possible to see how next non-invasive insulin product coming to the market based on microsphere technology can be transformed into an industrial scale, and keep its availability in a sustainable concept.

Declaration of interest

This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal (PTDC/SAU-FCF/104492/2008 and grants SFRH/BPD/35996/2007).

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Migdalis IN. Insulin analogs versus human insulin in type 2 diabetes. Diabetes Res Clin Pract 2011;93(Suppl 1):S102-S4
2. Gough SC. A review of human and analogue insulin trials. Diabetes Res Clin Pract 2007;77(1):1-15
3. MannKind Corp. Web Page. Available from: http://www.mannkindcorp.com/ [Last accessed 30 July 2012]
4. Richardson PC, Boss AH. Technosphere® insulin technology. Diabetes Technol Ther 2007;9(Suppl 1):S65-72
5. Al-Qadi S, Grenha A, Carrión-Recio D, et al. Microencapsulated chitosan nanoparticles for pulmonary protein delivery: in vivo evaluation of insulin-loaded formulations. J Control Release 2012;157(3):383-90
6. Cassidy J, Amin N, Marino M, et al. Insulin lung deposition and clearance following Technosphere® insulin inhalation powder administration. Pharm Res 2011;28(9):2157-64
7. Leone-Bay A, Baughman R, Smutney C, Kocinsky J. Innovation in drug delivery by inhalation. In: Publishing FF, editor. Orally inhaled & nasal drug products: innovations from major delivery system developers. ONdrugDelivery; East Sussex, United Kingdom: 2010. p. 4-7
8. Angelo R, Rousseau K, Grant M, et al. Technosphere insulin: defining the role of technosphere particles at the cellular level. J Diabetes Sci Technol 2009;3(3):545-54
9. Potocka E, Cassidy JP, Haworth P, et al. Pharmacokinetic characterization of the novel pulmonary delivery excipient fumaryl diketopiperazine. J Diabetes Sci Technol 2010;4(5):1164-73
10. Leone-Bay A, Grant M. Technosphere® technology: a platform for inhaled protein therapeutics. In: ONdrugDelivery, editor. Pulmonary delivery: innovative technologies breathing new life into inhalable therapeutics. ONdrugDelivery; East Sussex, United Kingdom: 2006. p. 8-11
11. Marino MT, Cassidy JP. The relationship between two insulin assays used to determine bioequivalence and dose proportionality of AFREZZA® insulin administered using a gen2 inhaler compared to a MedTone inhaler: simulation of clinical trials and actual data. Poster #141-RIA, presented at the Diabetes Technol Soc meeting; 2010
12. Pfützner A, Mann AE, Steiner SS. Technosphere™/Insulin—a new approach for effective delivery of human insulin via the pulmonary route. Diabetes Technol Ther 2004;4(5):589-94
13. Markey KA. MannKind Corp. Update report. Griffin Securities Equities Research; New York: 2011

•• **Last report created by MannKind Corp., showing the most relevant clinical data collected as the perspectives of the company about its inhaled insulin product, Afrezza®.**

14. Steiner S, Pfützner A, Wilson BR, et al. Technosphere/Insulin - proof of concept study with a new insulin formulation for pulmonary delivery. Exp Clin Endocr Diab 2002;110(1):17-21
15. Pfützner A, Forst T. Pulmonary insulin delivery by means of the Technosphere™ drug carrier mechanism. Expert Opin Drug Deliv 2005;2(6):1097-106
16. Rosenstock J, Lorber DL, Gnudi L, et al. Prandial inhaled insulin plus basal insulin glargine versus twice daily biaspart insulin for type 2 diabetes: a multicentre randomised trial. Lancet 2010;375(9733):2244-53
17. Potocka E, Baughman R, Derendorf H. Population pharmacokinetic model of human insulin following different routes of administration. J Clin Pharmacol 2011;51(7):1015-24
18. Heinemann L. Variability of insulin action: does it matter? Insulin 2008;3(1):37-45

• **Individual variability of insulin action, a rare focused issue, is briefly reviewed is this paper.**

19. Rave K, Heise T, Heinemann L, Boss AH. Inhaled Technosphere® insulin in comparison to subcutaneous regular human insulin: time action profile and variability in subjects with type 2 diabetes. J Diabetes Sci Technol 2008;2(2):2005-212
20. Rave K, Potocka E, Boss AH, et al. Pharmacokinetics and linear exposure of AFRESA™ compared with the subcutaneous injection of regular human insulin. Diabetes Obes Metab 2009;11(7):715-20
21. Raskin P, Heller S, Honka M, et al. Pulmonary function over 2 years in diabetic patients treated with prandial inhaled Technosphere Insulin or usual antidiabetes treatment: a randomized trial. Diabetes Obes Metab 2012;14(2):163-73

• **The most recent evidence of the inhaled insulin long-term safety collected from a clinical trial conducted at 220 sites with 1699 individuals.**

22. Rossiter A, Amin N, Harris R, et al. Pulmonary safety of inhaled Technosphere® Insulin therapy in adults with diabetes using high-resolution computerized tomography of the chest. Diabetologia 2009;52(S1):abstract #921
23. Skyler JS, Hollander PA, Jovanovic L, et al. Safety and efficacy of inhaled human insulin (Exubera®) during discontinuation and readministration of therapy in adults with type 1 diabetes: a 3-year randomized controlled trial. Diabetes Res Clin Pract 2008;82(2):238-46
24. Neumiller JJ, Campbell RK. Technosphere(R) insulin: an inhaled prandial insulin product. BioDrugs 2010;24(3):165-72
25. Forst T, Hohberg C, Schöndorf T, et al. Time-action profile and patient assessment of inhaled insulin via the Exubera device in comparison with subcutaneously injected insulin aspart via the FlexPen device. Diabetes Technol Ther 2009;11(2):87-92
26. Patton JS, Bukar JG, Eldon MA. Clinical Pharmacokinetics and Pharmacodynamics of inhaled insulin. Clin Pharmacokinet 2004;43(12):781-801
27. Rave K BS, Heinemann L, Sha S, et al. Time-action profile of inhaled insulin in comparison with subcutaneously injected insulin lispro and regular human insulin. Diabetes Care 2005;28(5):1077-82
28. Quattrin T, Bélanger A, Bohannon NJ, Schwartz SL; Group. EPIS. Efficacy and safety of inhaled insulin (Exubera) compared with subcutaneous insulin

therapy in patients with type 1 diabetes: results of a 6-month, randomized, comparative trial. Diabetes Care 2004;27(11):2622-7

29. Hollander PA, Blonde L, Rowe R, et al. Efficacy and safety of inhaled insulin (Exubera) compared with subcutaneous insulin therapy in patients with type 2 diabetes: results of a 6-month, randomized, comparative trial. Diabetes Care 2004;27(10):2356-62
30. Rosenstock J, Cefalu WT, Hollander PA, et al. Two-year pulmonary safety and efficacy of inhaled human insulin (Exubera) in adult patients with type 2 diabetes. Diabetes Care 2008;31(9):1723-8
31. Skyler JS, Jovanovic L, Klioze S, et al. Two-year safety and efficacy of inhaled human insulin (Exubera) in adult patients with type 1 diabetes. Diabetes Care 2007;30:579-85
32. Hollander P, Krasner A, Klioze S, et al. Body weight changes associated with insulin therapy: a retrospective pooled analysis of inhaled human insulin (Exubera 1) versus subcutaneous insulin in five controlled phase 3 trials. Diabetes Care 2007;30:2508-10
33. Rosenstock J, Foyt H, Klioze S, et al. Inhaled human insulin (Exubera®) therapy shows sustained efficacy and is well tolerated over a 2-year period in patients with type 2 diabetes (T2DM). Diabetes Metab 2006;55(S1):A109-OR
34. Alabraba V, Farnsworth A, Leigh R, et al. Exubera inhaled insulin in patients with type 1 and type 2 diabetes: the first 12 months. Diabetes Technol Ther 2009;11(7):427-30
35. Van Alfen-van der Velden A, Noordam C, De Galan BE, et al. Successful treatment of severe subcutaneous insulin resistance with inhaled insulin therapy. Pediatr Diabetes 2010;11(6):380-2
36. Hollander PA, Cefalu WT, Mitnick M, et al. Titration of inhaled human insulin (Exubera) in a treat-to-target regimen for patients with type 2 diabetes. Diabetes Technol Ther 2010;12(3):185-91
37. Harper NJ, Gray S, De Groot J, et al. The design and performance of the exubera pulmonary insulin delivery system. Diabetes Technol Ther 2007;9(Suppl 1):S16-27
38. Rosenstock J, Cappelleri J, Bolinder B, Gerber R. Patient satisfaction and glycemic control after 1 year with inhaled insulin (Exubera) in patients with type 1 or type 2 diabetes. Diabetes Care 2004;27(6):1318-23
39. Gerber RA CJ, Kourides IA, Gelfand RA. Treatment satisfaction with inhaled insulin in patients with type 1 diabetes: a randomized controlled trial. Diabetes Care 2001;24(9):1556-9
40. Wollmer P, Pieber TR, Gall MA, Brunton S. Delivering needle-free insulin using AERx® iDMS (Insulin Diabetes Management System) technology. Diabetes Technol Ther 2007;9(Suppl 1):S57-64
41. Cramer JA, Okikawa J, Bellaire S, Clauson P. Compliance with inhaled insulin treatment using the AERx iDMS insulin diabetes management system. Diabetes Technol Ther 2004;6(6):800-7
42. Mudaliar S. Inhaled insulin using AERx insulin diabetes management system (AERx iDMS). Expert Opin Investig Drugs 2007;16(10):1673-81
43. Brunner GA, Balent B, Ellmerer M, et al. Dose-response relation of liquid aerosol inhaled insulin in type I diabetic patients. Diabetologia 2001;44(3):305-8
44. Moses RG, Bartley P, Lunt H, et al. Safety and efficacy of inhaled insulin (AERx® iDMS1) compared with subcutaneous insulin therapy in patients with type 1 diabetes: 1-year data from a randomized, parallel group trial. Diabet Med 2009;26(3):260-7
45. Hermansen K, Rönnemaa T, Petersen AH, et al. Intensive therapy with inhaled insulin via the AERx insulin diabetes management system: a 12-week proof-of-concept trial in patients with type 2 diabetes. Diabetes Care 2004;27(1):162-7
46. Rosenstock J, Muchmore D, Swanson D, Schmitke J. AIR inhaled insulin system: a novel insulin-delivery system for patients with diabetes. Expert Rev Med Devices 2007;4(5):683-92
47. Ellis SL, Gemperline KA, Garg SK. Review of phase 2 studies utilizing the AIR particle technology in the delivery of human insulin inhalation powder versus subcutaneous regular or lispro insulin in subjects with type 1 or type 2 diabetes. Diabetes Technol Ther 2007(Suppl 1):S48-56
48. Muchmore DB, Silverman B, De La Peña A, Tobian J. The AIR inhaled insulin system: system components and pharmacokinetic/glucodynamic data. Diabetes Technol Ther 2007(Suppl 1):S41-7
49. de La Peña A, Seger M, Rave K, et al. AIR insulin capsules of different dose strengths may be combined to yield equivalent pharmacokinetics and glucodynamics. Diabetes Technol Ther 2009(Suppl 2):S75-80
50. Gross JL, Nakano M, Colon-Vega G, et al. Initiation of prandial insulin therapy with AIR inhaled insulin or insulin lispro in patients with type 2 diabetes: a randomized noninferiority trial. Diabetes Technol Ther 2009(Suppl 2):S27-34
51. Gross JL, Nakano M, Colon-Vega G, et al. Two-year efficacy and safety of AIR inhaled insulin in patients with type 1 diabetes: an open-label randomized controlled trial. Diabetes Technol Ther 2009(Suppl 2):S5-16
52. Bergenstal RM, Freemantle N, Leyk M, et al. Does availability of AIR insulin increase insulin use and improve glycemic control in patients with type 2 diabetes? Diabetes Technol Ther 2009;11(Suppl 2):S45-52
53. Hayes RP, Muchmore D, Schmitke J. Effect of inhaled insulin on patient-reported outcomes and treatment preference in patients with type 1 diabetes. Curr Med Res Opin 2007;23(2):435-42
54. Kapitza C HT, Fishman RS, Shapiro DA, et al. Impact of particle size and aerosolization time on the metabolic effect of an inhaled insulin aerosol. Diabetes Technol Ther 2004;6(2):119-27
55. Kim D, Mudaliar S, Chinnapongse S, et al. Dose-response relationships of inhaled insulin delivered via the aerodose insulin inhaler and subcutaneously injected insulin in patients with type 2 diabetes. Diabetes Care 2003;26(10):2842-7
56. Perera AD, Kapitza C, Nosek L, et al. Absorption and metabolic effect of inhaled insulin intrapatient variability after inhalation via the aerodose insulin inhaler in patients with type 2 diabetes. Diabetes Care 2002;25(12):2276-81
57. Baxter Presents Phase I Inhaled Insulin Study Results at Respiratory Drug Delivery Conference. Baxter

International, Inc. France. 2007. Available from: www.baxter.com/press_room/press_releases/2007/04-20-07-promaxx.html [Last accessed 18 September 2012]

58. White S, Brown LR, McGeehan J, et al. The science and technology of the PROMAXX biotechnology platform. RDD Eur 2007;1:51-8

59. Rashba-Step J. Efficient pulmonary delivery of biological molecules as PROMAXX microspheres. In: ONdrugDelivery, editor. Pulmonary drug delivery: new perspectives on inhalers. ONdrugDelivery; Hove: 2007. p. 21-4

60. Heise T, Brugger A, Cook C, et al. PROMAXX® inhaled insulin: safe and efficacious administration with a commercially available dry powder inhaler. Diabetes Obes Metab 2009;11(5):455-9

61. Siekmeir R, Scheuch G. Inhaled Insulin - does it become reality? J Physiol Pharmacol 2008;59(6):81-113

62. Stote R, Miller M, Marbury T, et al. Enhanced absorption of Nasulin™, an ultrarapid-acting intranasal insulin formulation, using single nostril administration in normal subjects. J Diabetes Sci Technol 2011;5(1):113-19

63. Leary AC, Stote RM, Breedt HJ, et al. Pharmacokinetics and pharmacodynamics of intranasal insulin administered to healthy subjects in escalating doses. Diabetes Technol Ther 2005;7(1):113-19

64. Leary AC, Stote RM, Cussen K, et al. Pharmacokinetics and pharmacodynamics of intranasal insulin administered to patients with type 1 diabetes: a preliminary study. Diabetes Technol Ther 2006;8(1):81-8

65. Stote R, Marbury T, Shi L, et al. Comparison pharmacokinetics of two concentrations (0.7% and 1.0%) of Nasulin, an ultra-rapid-acting intranasal insulin formulation. J Diabetes Sci Technol 2010;4(3):603-9

66. Leary AC, Dowling M, Cussen K, et al. Pharmacokinetics and pharmacodynamics of intranasal insulin spray (Nasulin™) administered to healthy male volunteers: influence of the nasal cycle. J Diabetes Sci Technol 2008;2(6):1054-60

67. CPEX Pharmaceuticals, Inc. Form 10-K Annual report: United States Securities and Exchange Commission. Washington, D.C. 20549. 2010.Available from: http://www.faqs.org/sec-filings/110331/CPEX-Pharmaceuticals-Inc_10-K/ [Last accessed 30 July 2012]

68. Illum L. Nasal drug delivery — recent developments and future prospects. J Control Release 2012;161(2):254-63

69. CPEX Pharmaceuticals Web Page. Available from: http://www.cpexpharma.com [Last accessed 30 July 2012]

70. Marina Biotech Web Page. Available from: http://www.marinabio.com/ [Last accessed 30 July 2012]

71. Benedict C, Frey Ii WH, Schiöth HB, et al. Intranasal insulin as a therapeutic option in the treatment of cognitive impairments. Exp Gerontol 2011;46(2–3):112-15

72. Schiöth HB, Frey WH, Brooks SJ, Benedict C. Insulin to treat Alzheimer's disease: just follow your nose? Expert Rev Clin Pharmacol 2011;5(1):17-20

73. Craft S, Baker LD, Montine TJ, et al. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. Arch Neurol 2012;69(1):29-38

74. Arbit E, Kidron M. Oral insulin: the rationale for this approach and current developments. J Diabetes Sci Tech 2009;3(3):562-7

75. Woitiski CB, Sarmento B, Carvalho RA, et al. Facilitated nanoscale delivery of insulin across intestinal membrane models. Int J Pharm 2011;412(1–2):123-31

76. Diabetology Web Page. Available from: http://www.diabetology.co.uk/ [Last accessed 8 July 2012]

77. Luzio SD, Dunseath G, Lockett A, et al. The glucose lowering effect of an oral insulin (Capsulin) during an isoglycaemic clamp study in persons with type 2 diabetes. Diabetes Obes Metab 2010;12(1):82-7

• **An important study where PK and PD profile of Capsulin was evaluated and compared with SC regular human insulin in T2DM patients.**

78. Sabetsky V, Ekblom J. Insulin: a new era for an old hormone. Pharmacol Res 2010;61(1):1-4

79. Oramed Web Page. Available from: http://www.oramed.com/ [Last accessed 8 July 2012]

80. Eldor R, Arbit E, Miteva Y, et al. Oral Insulin: Type I Diabetes (T1DM) Patient Response Upon Preprandial Administration. American Diabetes Association. 70th Scientific Session; 2010

81. Kidron M, Eldor R, Miteva Y, et al. Extended exposure to an oral insulin formulation yields decreased insulin secretion in type II diabetes subjects. Diabetes Technology Meeting; 2010

82. Emisphere Technologies Web Page. Available from: http://www.emisphere.com/index.html [Last accessed 8 July 2012]

83. Park K, Kwon IC, Park K. Oral protein delivery: current status and future prospect. React Funct Polym 2011;71(3):280-7

•• **This recent paper describes the several strategies of oral insulin delivery systems and current oral protein delivery technologies under development.**

84. Hoffman A, Qadri B. Eligen insulin - a system for the oral delivery of insulin for diabetes. IDrugs 2008;11(6):433-41

85. Emisphere Technologies Annual Report and Proxy. 2011.Available from: http://www.annualreports.com/HostedData/AnnualReports/PDF/EMIS2011.pdf [Last accessed 26 July 2012]

86. BOWS Pharmaceuticals AG Web Page. Available from: http://www.bowspharma.com/ [Last accessed 8 July 2012]

87. ClinicTrials.gov Web Page. Available from: http://clinicaltrials.gov/ [Last accessed 9 August 2012]

88. Merrion Pharmaceuticals Web Page. Available from: http://www.merrionpharma.com/ [Last accessed 8 July 2012]

89. Clinic Trials at Novo Nordisk Web Page. Available from: http://www.novonordisk-trials.com/ [Last accessed 25 July 2012]

90. Diasome Pharmaceuticals Web Page. Available from: http://www.diasome.com/ [Last accessed 9 July 2012]

91. Geho WB, Geho HC, Lau JR, Gana T. Hepatic-directed vesicle insulin: a review of formulation development and preclinical evaluation. J Diabetes Sci Technol 2009;3(6):1451-9

92. Schwartz S, Geho B, Rosenberg L, Lau J. Single-Blind, Placebo-Controlled, Dose-Ranging Trial of Oral

HDV-Insulin in Patients With Type 2 Diabetes Mellitus. Conshohocken, PA: Diasome Pharmaceuticals and San Antonio, TX: Diabetes Glandular Research Center. 2008.Available from: http://www.diasome.com/docs/FINAL%202009%20ADA%20Poster.pdf [Last accessed 30 July 2012]

93. Schwartz S, Geho B, Rosenberg L, Lau J. A Two-Week Randomized Active Comparator Study of Two HDV-Insulin Routes (SC and Oral) and SC Human Insulin in Patients With Type 1 Diabetes Mellitus (417-P). Poster session presented at: 68th Annual American Diabetes Association Meeting; 6 – 10 Jun 2008; San Francisco, Ca

94. Biocon Biopharmaceuticals Web Page. Available from: http://www.biocon.com/ Last [accessed 9 July 2012]

95. Iyer H, Khedkar A, Verma M. Oral insulin – a review of current status. Diabetes Obes Metab 2010;12(3):179-85

96. Access Pharmaceuticals, Inc. Web Page. Available from: http://www.accesspharma.com [Last accessed 1 August 2012]

97. Heinemann L. New ways of insulin delivery. Int J Clin Pract 2012;66:35-9

98. Generex Biotechnology Corp. Web Page. Available from: http://www.generex.com/index.php [Last accessed 23 July 2012]

99. Khafagy E-S, Morishita M, Onuki Y, Takayama K. Current challenges in non-invasive insulin delivery systems: a comparative review. Adv Drug Deliv Rev 2007;59:1521-46

100. Kumria R, Goomber G. Emerging trends in insulin delivery: buccal route. J Diabetol 2011;2(1):1-9

101. Kinesh VP, Neelam DP, Punit BP, et al. Novel approaches for oral delivery of insulin and current status of oral insulin products. IJPSN 2010;3(3):1057-64

102. Monosol Rx Web Page. Available from: http://www.monosolrx.com/ [Last accessed 3 August 20102]

103. Midatech Ltd. Available from: http://www.midatechgroup.com/ [Last accessed 3 August 2012]

104. Zubaerova DK, Larionova NI. Noninvasive insulin delivery systems. biochemistry (Moscow) supplement series B. Biomed Chem 2008;2(4):346-55

105. Jain A, Dhote V, Dhongade H, et al. Microneedles: a revolution of transdermal drug delivery. PR 2008;6(3)

106. Shivanand P, Binal P, Viral D. Microneedles: progress in developing new technology for painless drug delivery. IJPTR 2009;1(4):1279-82

107. Nordquist L, Roxhed N, Griss P, Stemme G. Novel microneedle patches for active insulin delivery are efficient in maintaining glycaemic control: an initial comparison with subcutaneous administration. PR 2007;24(7):1381-8

108. Silpi C, Manish B, Kumar TR. Microneedles in transdermal drug delivery: an unique painless option. IRJP 2011;2(4):72-8

109. Drug Delivery in Diabetes: making Effective Treatment Tolerable. ONdrugDelivery Web Page. Available from: http://www.ondrugdelivery.com/publications/diabetes.pdf [Last accessed 18 July 2012]

110. Shivanand P, Jigisha P, Kinjal P. Various emerging technologies in insulin delivery system. IJPRD 2009;1(8):1-6

111. Anhalt H, Bohannon NJV. Insulin patch pumps: their development and future in closed-loop systems. Diabetes Technology & Therapeutics 2010;12(S1):S-51-S-8

112. Valeritas, Inc. Web Page. Available from: http://www.valeritas.com/ [Last accessed 9 August 2012]

113. V-Go Disposable Insulin Delivery Device. Valeritas, Inc. Available from: http://www.go-vgo.com/ [Last accessed 30 July 2012]

114. Amin A, Shah T, Patel J, Gajjar A. Non Invasive Insulin - Annual Update on Non-Invasive Insulin Delivery Technologies. Drug Deliv Technol 2007;7(3):43-8.Available from: http://www.drugdeliverytech-online.com/drugdelivery/200803/?pg=43#pg43 [Last accessed 30 July 2012]

115. Chen H, Zhu H, Zheng J, et al. Iontophoresis-driven penetration of nanovesicles through microneedle-induced skin microchannels for enhancing transdermal delivery of insulin. J Control Release 2009;139:63-72

116. Polat BE, Hart D, Langer R, Blankschtein D. Ultrasound-mediated transdermal drug delivery: mechanisms, scope, and emerging trends. J Control Release 2011;152(3):330-48

117. Ogura M, Paliwal S, Mitragotri S. Low-frequency sonophoresis: current status and future prospects. Adv Drug Deliv Rev 2008;60(10):1218-23

118. Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. Science 1995;269(5225):850-3

119. Tezel A, Sens A, Tuchscherer J, Mitragotri S. Frequency dependence of sonophoresis. Pharm Res 2001;18(12):1694-700

120. Saroha K, Sharma B, Yadav B. Sonophoresis: an advanced tool in transdermal drug delivery system. IJCPR 2011;3(3):89-97

121. Polat BE, Blankschtein D, Langer R. Low-frequency sonophoresis: application to the transdermal delivery of macromolecules and hydrophilic drugs. Expet Opin Drug Deliv 2010;7(12):1415-32

122. Mitragotri S. Synergistic effect of enhancers for transdermal drug delivery. Pharm Res 2000;17(11):1354-9

123. Transdermal Specialties, Inc. Web Page. Available from: http://www.transdermalspecialties.com [Last accessed 19 September 2012]

124. Transdermal Specialties, Inc. 2012 Offering documents. Available from: http://www.transdermalspecialties.com/pdf/TSI_2012_ppm.pdf [Last accessed 9 August 2012]

125. GlobeNewswire Web Page. Available from: http://www.globenewswire.com/ [Last accessed at 9 August 2012]

126. Kulkarni PR, Yadav JD, Vaidya KA, Gandhi PP. Transferosomes: an emerging tool for transdermal drug delivery. IJPSR 2011;2(4):735-41

127. Wadhwa S, Rawat S, Singhal S, et al. Transferosome: a versatile tool for transdermal drug delivery. JPR 2012;5(1):610-15

128. Kapsner P, Bergenstal R, Rendell M, et al. Comparative efficacy and safety of Technosphere® insulin and a rapid-acting analog both given with glargine in subjects with T1DM in a 52-week study. Diabetologia 2009;52(S1):abstract #982

129. Rosenstock J, Bergenstal R, Defronzo RA, et al. Efficacy and safety of Technosphere inhaled insulin compared with Technosphere powder placebo in insulin-naive type 2 diabetes suboptimally controlled with oral agents. Diabetes Care 2008;31(11):2177-82

130. Tack CJ, Christov V, Galan BE, et al. Randomized forced titration to different doses of Technosphere® insulin demonstrates reduction in postprandial glucose excursions and hemoglobin A1c in patients with type 2 diabetes. J Diabetes Sci Technol 2008;2(1):47-57

131. Petrucci R, Amin N, Lovertin P, et al. Pulmonary function tests remain similar in patients who received technosphere insulin and in patients currently receiving standard antidiabetic therapy [abstract #919]. Diabetologia 2009;52(S1):S361

132. Peyrot M, Rubin RR. Patient-reported outcomes in adults with type 2 diabetes using mealtime inhaled technosphere insulin and basal insulin versus premixed insulin. Diabetes Technol Ther 2011;13(12):1201-6

133. Peyrot M, Rubin RR. Effect of technosphere inhaled insulin on quality of life and treatment satisfaction. Diabetes Technol Ther 2010;12(1):49-55

134. Heinemann L. The failure of exubera: are we beating a dead horse? J Diabetes Sci Technol 2008;2(3):518-29

•• **A refreshing overview about inhaled insulin where several viewpoints (patients, diabetologists, scientists, pharmaceutical industry, healthcare payers and politicians) are exposed.**

135. Nektar Therapeutics Web Page. Available from: http://www.nektar.com/ Last [accessed 30 July 2012]

136. Aradigm Corp. website. Available from: http://www.aradigm.com [Last accessed 30 July 2012]

137. Alkermes Web Page. Available from: http://www.alkermes.com/ [Last accessed 30 July 2012]

138. Aerogen Web Page. Available from: http://www.aerogen.com/ [Last accessed 30 July 2012]

139. Aerogen, Inc. Form 10-K Annual report: United States Securities and Exchange Comission. Washington, D.C. 20549. 2005.Available from: http://www.getfilings.com/o0001104659-05-016717.html [Last accessed 30 July 2012]

140. Abbott Web Page. Available from: http://www.abbott.com/ [Last accessed 30 July 2012]

141. Vectura Web Page. Available from: http://www.vectura.com/ [Last accessed 30 July 2010]

142. MicroDose Therapeutx Web Page. Available from: http://mdtx.com/ [Last accessed 30 July 2012]

143. Siekmeier R, Scheuch G. Diabetes treatment by inhalation of insulin - shine and decline of a novel type of therapy. In: Esquinas AM, editor. Applied technologies in pulmonary medicine. Karger; Basel: 2011. p. 77-83

144. Coremed Web Page. Available from: http://www.coremedusa.com/ [Last accessed 30 July 2012]

145. BioSante Pharmaceuticals Web Page. Available from: http://www.biosantepharma.com/ [Last accessed 30 July 2012]

146. Biopharmaceutiques Web Page. Available from: http://www.biopharmaceutiques.com/ [Last accessed 8 July 2012]

Affiliation

Sandra Soares[1], Ana Costa[1] & Bruno Sarmento[†1,2,3]
[†]Author for correspondence
[1]CICS, Department of Pharmaceutical Sciences, Instituto Superior de Ciências da Saúde-Norte, CESPU, Gandra, Portugal
[2]University of Porto, Faculty of Pharmacy, Department of Pharmaceutical Technology, Porto, Portugal
Tel: +351 963078375;
E-mail: bruno.sarmento@iscsn.cespu.pt
[3]University of Porto, INEB, Institute of Biomedical Engineering, Porto, Portugal