Various Non-Injectable Delivery Systems for the Treatment of Diabetes Mellitus

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Abstract: Diabetes mellitus (diabetes) is suffered by more than 180 million people and is responsible for approximately 2.9 million deaths each year. This mortality rate is expected to increase by 50 % in the next decade. Due to the inconvenience of the traditional treatment of diabetes by subcutaneous administration of insulin injection, various attempts are made in the production, purification, formulation and methods of delivery of insulin. However, despite advances in recent years, these attempts have met with limited success. Various alternative routes such as rectal, ocular, nasal, pulmonary and oral have been exploited. The pulmonary route offers great potential for the delivery of polypeptide drugs due to the large surface area for insulin absorption in the respiratory tract. But due to its low bioavailability, oral route is intensely investigated for the insulin delivery. Microencapsulation, as one of the delivery systems utilising oral route, has shown some potential progress in insulin delivery; though it is at an early stage yet it has proved to be quite encouraging providing new less toxic immunosuppressive agents. Microencapsulation may prove to be an attractive delivery system for controlled release of insulin and beneficial for therapeutic, bio-efficient and bio-effective drug delivery. In this review we discuss the possible alternative routes for insulin delivery (ocular, nasal, pulmonary and oral) and advantages and disadvantages of each. Furthermore we consider the different drug delivery strategies available (aerosols, dry powder inhalers, synthetic beta cells, hydrogels and microcapsules) and their current and potential applications with respect to the different insulin delivery routes.

Key Words: Insulin delivery, hydrogels, aerosols, microcapsules, dry powder inhalers.

INTRODUCTION

Diabetes mellitus (commonly known as diabetes) is one of the leading causes of mortality. Recent World Health Organisation (WHO) estimations indicate that worldwide number of deaths attributable to diabetes is around 2.9 million each year (Fig. **1**) and is predicted to increase by more than 50% in the next 10 years. At present, more than 180 million people worldwide have diabetes and most people are middle-aged (45-64) and not elderly (65+), especially in low and middle income countries such as India, Bangladesh, China, Indonesia [1].

In diabetes mellitus, insulin levels are too low to remove glucose from the plasma leading to hyperglycaemia. Diabetes is a life threatening condition which can lead to chronic disorders such as diabetic polyneuropathy [2] (peripheral neuropathy, autonomic neuropathy, proximal neuropathy and focal neuropathy), retinopathy [3] (macular oedema) and nephropathy [4]. Diabetes is mainly characterised by hyperglycaemia due to disregulation of glucose metabolism. In normal individuals, the increase in the blood glucose levels triggers the secretion of insulin by pancreatic islet beta cells **(**Fig. **2)**. The insulin binds to insulin receptors located on cells, and hence signals them to increase the rate of glucose uptake from the plasma into the cells. As the blood glucose returns to normal levels, the amount of insulin in the blood again drops. Therefore, in the absence of insulin, blood glucose levels would rise to dangerously high levels, often resulting in death. The WHO recognises two main forms of diabetes mellitus: *type 1 and type 2*.

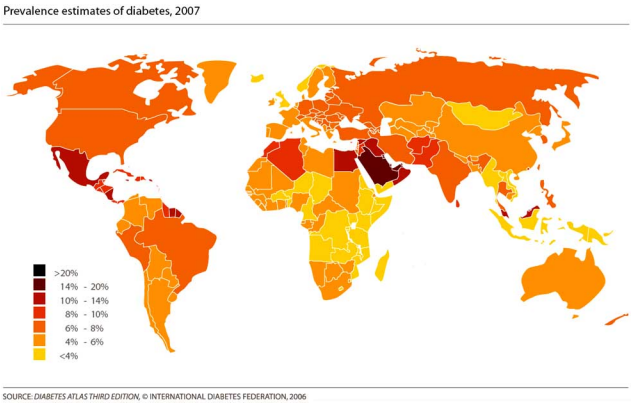

Fig. (1). World map showing the prevalence estimation of diabetes. The world map shows the comparative prevalence (%) estimation of diabetes across the world by the year 2007 and year 2025. The percentage of the number of people with diabetes increases with the colour intensity. Data reproduced from: *Diabetes Atlas*, third edition © International Diabetes Federation, 2006.

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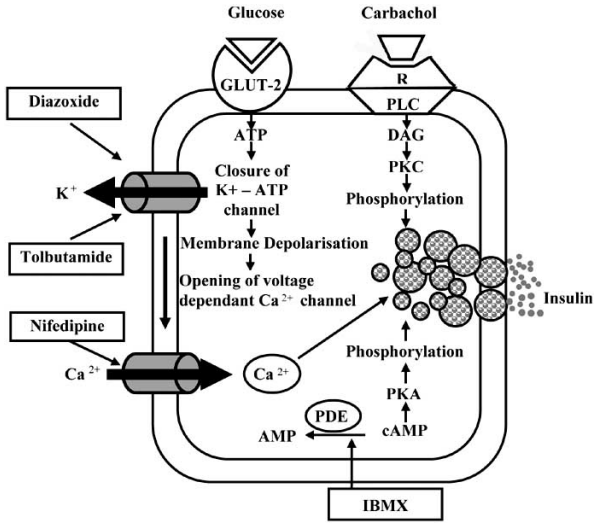

Fig. (2). Diagrammatic summary of the documented actions of glucose, cAMP, K1, and Ca21 on insulin secretion. Effects of known pharmacological regulators of insulin release are indicated. DAG, diacylglycerol; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C. Adapted from [5].

Type 1 diabetes or insulin-dependent diabetes mellitus (IDDM) is characterised by the lack of insulin production and usually develops in childhood. It is often categorised as autoimmune disease [6]. The immune system makes antibodies that attach to the beta cells in the pancreas and destroys them, thus stopping insulin production. Hence, the patient becomes dependent on the external sources of insulin for survival.

Type 2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM), generally occurs in middle aged people. It is the far more common form of diabetes, comprising 90% of the worldwide population of diabetic patients. Although these patients produce normal (or even high) levels of insulin in their blood and the insulin attaches normally to the receptors on cells, they exhibit a low rate of cellular uptake of glucose in response to insulin.

The treatment of diabetes mellitus is both difficult and tedious. The desire and demand for a novel drug delivery system to produce insulin in response to blood glucose levels has been increasing since the discovery of insulin in 1921 [7]. After 60 years of research, the first commercially available insulin pumps appeared in the market in the 1980. Since then an extensive research has been carried out to develop drug delivery systems for specifically targeted parts of the body controlled by means of either physiological or chemical triggers.

Traditionally, diabetes has been treated by regularly injecting the patients with insulin. The glucose level of the patient is monitored and when hyperglycaemia occurs insulin is injected by the subcutaneous route. The saline solution of insulin is gradually absorbed into the bloodstream *via* the dermal capillaries, where it reaches its maximum activity at 2 to 3 hours following injection. Certain slow-acting formulations of insulin such as Lente insulin show an even more prolonged effect [8]. However, the subcutaneous administration of insulin is quite inconvenient and painful. Additionally, its overdose may lead to hyperinsulinaemia as insulin is administered in a non-physiological way, targeting mainly extrahepatic insulin-dependent tissues [9]. Insulin delivery is a vast area of research; various factors such as administration routes, form of delivered insulin, dose, *etc* affect the efficacy of insulin. Among the other non-invasive routes of insulin administration, rectal route has been investigated. The water solubility of insulin has been exploited for the delivery of the drug directly in to the general circulation *via* lymphatic uptake. Barichello *et al.* [10] proposed the rectal administration as a potential vehicle for the delivery of insulin. Rectal administration of insulin offers some advantages such as reduced polypeptide degradation and partial avoidance of hepatic first pass metabolism. Due to its inconsistent insulin absorption and low bioavailability (4–10 %) this route is not well accepted as compared to the other routes of administration [11]. Different absorption enhancers have been used such as oleic acid, linolenic acid, linoleic acid and enamine derivatives for the rectal delivery but did not prove to be an acceptable alternative route [12]. Therefore, various attempts have been made to replace the local discomfort and multiple administrations of insulin injections by non-injectable routes. Unfortunately due to time limitation, this review paper focuses only on the various non-injectable routes of insulin administration (ocular, nasal, pulmonary and oral) and various strategies available (aerosols, dry powder inhalers, synthetic beta cells, hydrogels and microcapsules) for the treatment of diabetes mellitus.

NON-INJECTABLE METHODS OF INSULIN DELIVERY

Ocular Route

Another non-invasive route of insulin delivery is ocular route where insulin can be delivered into the systemic circulation through the instillation of eye drops, for which the major absorption site of insulin is located in the nasal cavity [13]. Systemic delivery of insulin through the ocular route offers many advantages such as convenient administration of drug as eye drops, accurate dose due to the fact that eyes can hold only one drop of ophthalmic solution regardless of the volume instilled, faster absorption, avoidance of hepatic first pass metabolism, more economical than injections as does not require syringes and needles [14]. Formulations generally contain insulin and enhancers such as deoxycholic acid, decamethonium bromide, polyethylene ethers. The delivery system utilises the lachrymal system to deliver the drug to its absorption site. Under normal conditions, lachrymal system produces and drains tears into the nasal cavity at a constant rate. However, if droplets containing insulin are introduced into the lower conjunctival sac, the lachrymal system increases its tear clearance rate and hence reduces the amount and contact time of insulin in the nasal cavity. Therefore, the therapeutic efficacy of insulin eye drop formulations may be low and highly variable. However, it has been proposed that an optimum insulin efficacy can be obtained by using the appropriate ocular insert [15].

Nasal Route

Nasal drug administration has also been used as an alternative route for the insulin administration. Various factors such as large surface area, porous endothelial membrane,

high total blood flow, avoidance of first-pass metabolism, and ready accessibility; offer a better absorption site for insulin in nasal cavity [16]. The advantages of this drug delivery system include controlling the rate of drug clearance from the nasal cavity and protecting the drug from enzymatic degradation in nasal secretions. Bioavailability of the drug formulations such as microspheres, liposomes and gels, largely depends on their bioadhesive properties which increase the contact time for absorption [17]. In addition to nasal airflow characteristics, many other factors such as physicochemical properties of the particles (time, dose and frequency of administration) and type, volume and concentration of insulin or absorption enhancers; influence the bioavailability of intranasal insulin [18]. In order to increase the absorption of insulin across the nasal mucosa, many absorption enhancers have been tested including bile salts, fusidic acid derivatives, surfactants, phospholipids and cyclodextrins [19]. Pharmacokinetic studies [20-22] in healthy and diabetic individuals showed that nasal delivery of insulin resulted in rapid increase and quick decrease of the serum insulin concentrations, reaching peak concentration at approximately 10–25 minutes following administration. The profile seemed to be favourable for prandial insulin replacement and have led to a number of studies estimating the efficacy of nasal insulin in reducing the postprandial glycaemia excursions in both *type 1* and *type 2* diabetic patients.

It has been reported that intranasal insulin administration has many side effects such as nasal irritation and damage of the nasal mucosa and mucociliary function. Studies [23-25] reported that insulin preparations for nasal administration containing sodium glycolate or sodium taurofusidate as an absorption promoter caused irritation on the nasal mucosa. Some of other disadvantages include increased systolic, diastolic and mean arterial blood pressure [17]. Furthermore the maximum dose is limited to about 150 µl [25]. Although the nasal route may prove to be a potential method for insulin delivery, there are several limitations with its use; thus the delivery system needs to be further studied before it can be safely and effectively used in clinical practice.

Oral Route

The oral route for insulin administration is of particular interest as in general oral drug administration results in less pain, greater convenience, higher compliance and reduced infection risk as compared to subcutaneous injections [26]. An extensive amount of research has been carried out to improve buccal absorption as it offers an excellent accessibility and a large area for absorption with little proteolytic activity. Some recent pharmacokinetics and pharmacodynamics studies on *type 1* diabetic patients, have illustrated that the oral insulin spray showed peak insulin concentrations in relatively shorter time periods, a more rapid onset of action and faster run off as compared to regular subcutaneous insulin [27]. Some other studies on *type 1* diabetic patients on multiple daily insulin injections and in insulin-treated *type 2* diabetic patients showed that the oral insulin was effective in controlling postprandial glucose levels [28]. However, there are disadvantages associated with this route of administration such as low bioavailability due to relatively low passage of active agents across the mucosal epithelium, rapid insulin degradation due to action of digestive enzymes in the GI tract, enzymatic proteolysis and acidic degradation of orally administered insulin in the stomach [29]. Various approaches have been made to increase the buccal penetration using permeation enhancers [30, 31], protease inhibitors [32], enteric coatings [33] and (bio) polymer micro-/ nano-sphere formulations [34, 35].

Pulmonary Route

Pulmonary drug delivery systems offer an alternate and a better route of insulin administration as compared to the other non-invasive routes. Pulmonary delivery of insulin was first reported in 1925 [36], very shortly after the first clinical use of insulin [37]. They are designed to be inhaled by patients in the form of drug dispersions where the active drug within the dispersion can reach the lung. They get readily absorbed through the alveoli directly into blood stream because of large surface area (~140 m^2 in adults), high permeability, and vast vascularisation of the lung; and hence provide higher drug efficacy [38]. Other advantages include relatively simple self-administration, no first-pass liver effect of the absorbed insulin, reduced enzymatic activity and pH mediated drug degradation. Due to the disadvantages of intranasal delivery of insulin as mentioned earlier, pulmonary route proves to be a promising non - injectable route for insulin delivery. One of the most important advantage of this route is higher absorption; the surface area for absorption of the alveolar region of the lung is relatively higher (~ 140 m^2) as compared to that of the nasal absorption region (~ 150 cm^2) [39]. The other advantage of the pulmonary administration is that drugs have longer residence time in the alveolar region of lungs due to minimal clearance mechanisms; as compared to the intranasal administration where the drug needs to be absorbed quickly (in approximately 15 to 20 minutes) or they are removed by the rapid mucociliary clearance mechanisms in the nose and if swallowed [40]. In addition, the alveolar region has an extremely thin (0.1 µm) and vesiculated cell barrier which enhances the absorption of drugs that are deposited there [41]. Pulmonary route has also proved to be more beneficial than the subcutaneous route. Some studies have shown that intrapulmonary delivery of nebulised insulin showed the average time to peak insulin levels between 50 and 60 minutes [42], while those injected subcutaneously, the average time to peak insulin levels was 144 minutes [43]. Commonly used devices for pulmonary insulin administration are metered dose inhalers (MDIs) and nebulisers which contain volatile chemical propellants such as hydrofluoroalkanes (HFAs) [44].

The major issue related to this route of insulin administration is bioavailability. The bioavailability of inhaled insulin is less than 20 %, lower than the subcutaneously administered insulin; thus, dosage requirements and cost per treatment are higher in comparison with insulin administered by subcutaneous injection. The route does not deliver the drug (in an appropriate formulation) effectively, deep into the lung in order to penetrate into the blood circulation. Besides offering a larger absorption area, the respiratory system is also designed as a series of filters which prevents environmental aerosols to get into the deep lung and to keep the lung surface clean, thus hindering in the efficient delivery of aerosolised insulin [45]. The oropharyngeal region and the bronchial tree act as filters to eliminate aerosol particles from the

inhaled air; the particles deposited on the ciliated epithelium of the bronchial tree are transported to the gastrointestinal tract. Therefore, this filtration poses a problem in the efficient delivery of drugs deep into the lungs. The deposition behaviour of aerosol particles in the respiratory tract is also driven by factors such as physical properties of the particle, chemical properties of the drug, and physiological (breathing pattern, pulmonary diseases, smoking) factors [46]. In addition, absorption kinetics of inhaled substances deposited in the lung periphery is also affected by their molecular weight, pH-value, electrical charge, solubility, and stability of the inhaled substance [47]. Inadequate consideration of these factors often leads to insufficient deposition and irreproducible amount of a drug in a predefined lung region by pulmonary administration.

One of the other potential drawbacks of aerosolised drug delivery systems includes the protein stability. During the aerosolisation process the liquid undergoes multiple passes that could compromise stability and increase the odds of contamination [48]. To maintain protein stability in the formulation; the dry powder formulations need buffers to maintain the pH, and surfactants to reduce protein aggregation during prolonged storage. Although, the long-term effects of intra-alveolar insulin deposition of inhaled insulin have not been fully elucidated, some studies [49] have shown that irrespective of the type of pulmonary delivery system, inhaled insulin produced more insulin antibodies compared to subcutaneous injection. Loss of 90 % bioavailability of insulin along with the above mentioned disadvantages associated with the route; has given a new direction for the development of therapies and strategies for insulin delivery through other routes of administration.

CURRENTLY AVAILABLE INSULIN THERAPY STRATEGIES

Aerosols

Although the delivery of insulin by inhalation has been most extensively studied, the inconvenience and discomfort has led to research into aerosols. Research has focused on developing medicaments for treating respiratory and nasal disorders by aerosolised drugs that could be delivered by either mouth or nose. These formulations are made effective by adding surface-active materials to the aerosolised solution being introduced to the respiratory tract. The controlled systemic or local delivery of insulin results in enhanced absorption, less nasal irritation and congestion. These aerosolised formulations are also used for the systemic delivery of other peptides and proteins which include calcitonin for Paget's disease and osteoporosis; leuprolide acetate for prostate cancer, breast cancer and infertility; growth hormone-releasing factor for the treatment of pituitary short stature; and morphine for analgesia [50]. Generally, the aerosol is formulated by suspending the drug as a finely divided powder in a liquefied gas known as a propellant such as chloroflourocarbons (CFCs). Surface active agents are also added to facilitate dispersion of the drug in the propellant, to prevent aggregation of the micronised drug particles (1 – 3 micron in diameter), and to improve lubrication of the valve. These formulations are then dispensed to the patients through pressurised metered dose inhalers (pMDIs). However, the use of CFCs is banned because of their adverse effect on the ozone layer. Therefore, they have been replaced by hydrofluoroalkanes (HFAs) which share similar desirable characteristics in terms of nonflammability, chemical stability, vapour pressure whilst being non-ozone depleting [51].

Some studies [52, 53] on aerosolised insulin delivery in diabetic patients have shown that bioavailability of an inhaled dose of aerosolised insulin was approximately 20% compared to those dose delivered by subcutaneous injection. Bioavailability studies [54] on smokers and non-smokers showed that smoking significantly affects the bioavailability of nebulised insulin aerosol. The report showed that the bioavailability of nebulised insulin was significantly higher in smokers. The possible explanation for the observation may be that smoking makes lungs more "leaky" by damaging the lung mucosa, hence, allowing more drug to enter the systemic circulation. However, no difference in the time to peak insulin levels was observed between smokers and non-smokers.

However, there are potential roadblocks in drug delivery (especially macromolecules) by aerosolised formulations such as protein denaturation during aerosolisation [55], excessive loss of inhaled drug in the oropharyngeal cavity [56], lack of reproducibility of therapeutic results owing to variations in breathing patterns [57]. Many factors affect the efficacy of insulin delivery to the lungs such as size, density, surface morphology, charge, solubility and hygroscopicity of the particles, the breathing pattern [58, 59], interstitial lung disease or airflow obstruction [60], smoking [61, 62], exercise [63, 64] and patients' ability to operate inhaler devices [65]. In addition, prolonged storage of proteins and peptides in the aqueous environments result in their instability, thus making their storage problematic [66]. Another limiting factor is the inefficient delivery of aerosolised insulin through jet-type nebulisers with and without spacer devices or holding chambers [67]. Most of the drug remains in the device and is not delivered to the lung, hence decreasing the bioavailability of insulin. Moreover, insulin delivery is inaccurate, with least precision, especially compared to insulin pumps capable of delivering tenths of a unit with precision [68]. Transport enhancing additives also produce additional adverse effects such as nasal irritation, rhinnorhea, cough, shortness of breath, sore throat and dry mouth; wherein, a mild cold or stuffiness may also alter the intended insulin dose change. Due to all these disadvantages, new formulations for insulin aerosols are being developed.

Dry Powder Inhalers

A significant progress in insulin delivery was achieved after realising the importance of aerosol dynamics in 1990s [69, 70] nearly 70 years after the initial attempts to deliver insulin as an inhaled aerosol in 1920s [36]. As discussed above, many drugs currently administered by inhalation are primarily liquid aerosol formulations which often lead to the instability of drugs, especially, proteins and peptides. In addition, due to the need to reformulate pMIDs to CFC-free systems using HFAs, dry powder inhalations (DPI) are becoming quite popular for pulmonary delivery. Conventionally, DPIs are used to deliver insulin by combining it with an appropriate absorption enhancer, to enhance the systemic

absorption of the drug; and introducing into the lung in the form of a powder of appropriate particle size (≤ 10 microns), where insulin readily enters the pulmonary circulation by absorption through the epithelial layer in the lower respiratory tract. These particles are produced by crystallising the powder and then milling to appropriate size for pulmonary delivery. An ideal DPI formulation should have uniform distribution, small dose variation, sufficient physical stability in the device before use and good performance in terms of emitted dose and fine particle fraction [71]. Therefore, powdered form of a drug supplies the optimum dose for maximum absorption in the lower respiratory tract, as compared to the other routes of delivery.

Many clinically tested and approved devices for dry powder formulations are available in the marketplace, such as Nektar / *Exebura*® device (Nektar Therapeutics Inc., San Carlos, CA, Aventis, Bridgewater, NJ, Pfizer, NY), or liquid aerosols formulations in the AERx® Insulin Diabetes Management System (Aradigm Corp., Hayward, CA, NovoNordisk A/S, Copenhagen, Denmark) and in the Aerodose® Inhaler (Aerogen Inc., Sunnyvale, CA, USA) [12]. Nektar / *Exebura*® devices supply powdered insulin to the lungs using compressed air prior to inhalation. The dry powder formulation consists of insulin (~ 60%) and excipients, such as mannitol which also acts as a stabiliser. The powdered insulin is dispersed in the form a blister (containing 1 - 3 mg of insulin) by the inhaler into an aerosol cloud held in a chamber. These aerosol clouds, containing insulin particles, are then inhaled by the patient at the beginning of a slow, deep breath during which draws air into the chamber and thus extruding the aerosol into the lungs. Some other delivery technologies use modified insulin particles for example in the AIR™ Pulmonary Drug Delivery System (Advanced Inhalation Research Alkermes, Cambridge, MA, Eli Lilley, Indianapolis) and Technosphere™ insulin (Mannkind Biopharmaceuticals, NY, USA). This form of insulin delivery, developed by Advanced Inhalation Research (AIR), uses a biodegradable polymer matrix comprised of phospholipids [72]. This formulation is easily aerosolised using simple, breath-activated dry powder inhalers. These large, porous, polymer particles containing insulin show less aggregation and less susceptibility towards phagocytosis.

Many clinical studies have shown that dry powdered formulation of insulin has proved to be effective in treating diabetes in comparison to the other means of insulin delivery. In a recent study of patients with *type 2* diabetes [73], oral therapy was not effective in controlling the glucose levels in the blood. However, treatment with the inhaled, powdered form of insulin proved to be beneficial in achieving adequate glycaemic control over a 3-month treatment period. The introduction of the inhaled insulin, however, increased the overall rate of hypoglycaemia and resulted in more frequent insulin-binding antibodies than in those patients who remained on combined oral treatment. In addition, those patients receiving insulin *via* the pulmonary route had greatest overall treatment satisfaction. Another study showed no difference in the overall glycaemic control in *type 2* diabetic patients, when treated with mixtures of soluble and NPH insulin with inhaled insulin, twice a day, prior to meals and ultralente insulin at bedtime for an extended period of 6 months [74]. Similar studies on patients with *type1* and *type2* diabetes carried out for two years revealed that glycaemic control and lung function tests (spirometry) remained significantly stable during the treatment [75].

The major disadvantage of the DPI is low bioavailability; as already mentioned the bioavailability is approximately 10% to 20% that of a subcutaneous dose. Primary reasons for such low efficiency are loss of insulin by several mechanisms such as adherence to the delivery device, deposition in the oropharynx and upper bronchial tree, exhalation of particles, breakdown by enzymes, and elimination by macrophages. The bioavailability may also vary among the insulin systems and patients; even dose varies non-uniformly in the same patient. One of the studies showed that the levels of insulin AERx varied in the same patients with asthma as compared to those in healthy controls [76].Various other physiological factors such as smoking, asthma and pulmonary diseases have also shown some effect on the non-uniformity of insulin dose. In a 6-month, randomised, comparative trial [77] on the patients with *type 1* diabetes, severe hypoglycemia was twice as frequent with insulin Exubera as with subcutaneous regular insulin. Some studies [78, 79] also reported the higher incidence of hypoglycemia with insulin Exubera than with oral hypoglycaemic agents. Another disadvantage associated with the system is the insulin-antibody binding. Several clinical trial studies [77-79] showed that during phase II and III trials, the prevalence of insulin antibodies was significantly higher in patients using insulin Exubera than in those receiving subcutaneous insulin. The levels of antibodies were higher in the patients with *type 1* diabetes than those with *type 2* diabetes.

Despite apparently providing a better and an effective system for insulin delivery, disadvantages such as poor control over powder crystallinity, shape, size, and size distribution, are also associated with the delivery system. Dry milling may produce high surface charge in partially amorphous particles, which in turn may lead to particle agglomeration. However, these problems may be overcome by improving the milling methods, such as carrying out milling process at elevated humidity. Particle engineering has shown an improved performance of dry powder aerosol systems by lowering the aerodynamic diameters of the particles, lowering particle density [80-83], altering shapes [84] and by creating rough surface (to increase the air drag force). Thus, development and manufacturing process thus becomes more complex and expensive. All these disadvantages have led the researchers to explore other delivery systems with higher bioavailability for the treatment of diabetes.

Synthetic Beta Cells

The glucose responsive release of insulin from beta cells is a complex event occurring during various stages including gene expression, post-translational modification and secretion. Mature insulin comprises of A and B polypeptide chains with disulfide bonds. Mature insulin (initial protein product) and insulin precursor known as preproinsulin, has the polypeptide structure of N-terminal signal sequence and C-terminal intervening sequence between A and B chains. C-terminal intervening sequence is cleaved during transport from the rough endoplasmic reticulum to form proinsulin.

Mature insulin is then released into the blood following the removal of residual 3 kDa fragment C-peptide by catalytic enzymes (endopeptidases). Mature insulin, stored in secretory granules, is released in response to elevated blood glucose levels [6].

Insulin is normally produced in and secreted by the beta cells of the islets of Langerhans in the pancreas. Autoimmune destruction of these pancreatic beta cells causes *Type I* diabetes [6]. As a consequence of partial or complete loss of beta cells, little or no insulin is secreted by the pancreas, resulting in higher blood glucose levels. Some success has been achieved by transplanting the pancreas in order to replace beta cell function. Besides being too expensive, the limited supply of primary human islets from heart-beating donors restricts the clinical application of this approach. However, there have been many other proposed alternatives for beta cell replacement such as bone marrow transplantation. It has been reported that *in vitro* culture of bone marrow stromal cells can differentiate at a low frequency into insulin-expressing cells [85]. A recent *in vivo* study using genetically tagged bone marrow cells when engrafted into pancreatic islets expressed beta cell markers [86]. However, for the other tissue stem cells, the potential usefulness of bone marrow stem cells depends on the ability of the cells to expand *in vitro* to clinically significant numbers.

Another method utilises non-islet cells comprising a genetic construct that has a coding sequence for a proinsulin expressible in the cells in response to glucose levels. Non-islet cells with genetic coding sequences are expressed for levels of proinsulin, in response to the elevated blood glucose levels, which in turn produces active insulin. Pluripotent stem-cells are considered to be the potential source of generating insulin-cells for beta cell replacement therapy. They offer benefits in comparison to the limited pancreatic donors in terms of providing limitless supply of physiologically competent substitute for primary human islets of Langerhans. Another advantage it offers over the exogenous insulin administration is the insulin secretion in response to blood glucose levels in the microenvironment.

Other alternative approaches for beta cell replacement include artificial beta cell production by engineering endocrine cells of the At-T-20 ACTH secreting cells [87]. This study showed that the stably transfected cell, At-T-20, was obtained by introducing cDNA encoding human insulin and the glucose transporter gene (GLUT-2) driven by the constitutive CMV promoter. The cell line was shown to express the correct isoform of glucokinase required for the expression of the proinsulin gene in response to blood glucose levels. Another approach by Laurance *et al*. [88] uses cell-lines in which insulin production is secretagogue-regulated.

Despite various new therapeutic possibilities for the treatment of *Type 1* diabetes and the potential gains, both clinical and commercial, there are still some problems in devising methods for the efficient and reliable production of functional insulin-secreting cells from pluripotent progenitors [89]. Besides this, these heterologous cells are recognised as foreign cells by the immune system. Thus, the cells have to be protected from immunoactive cells (*e.g.* T-cells and macrophages mediating the cytolytic processes). This can be done using physical immunoisolation approach; however, immunoisolation itself poses significant problems. Hence, the widespread application of islet transplantation depends on further improvements in immunosuppressive strategies, advances in the area of transplantation tolerance, increases in the longevity of islet transplants, and development of an unlimited source of beta cells.

Hydrogels

Another type of delivery system comprises of "hydrogels" as potential drugs carriers. This method uses a gelled network which traps the drug under certain conditions and then releases the active compound in a controlled manner by "swelling" or expanding inside specific tissues, thus allowing a higher concentration of the drug in a biodegradable format. Hydrogels are three-dimensional networks of hydrophilic polymers held together by cross-linking *via* covalent or ionic bonds and/ or secondary forces including hydrogen bonds or hydrophobic interactions. These interactions result in the reversible ionisation, which in turn initiates an osmotic pressure gradient causing the volume expansion or contraction of the hydrogel *via* the movement of water into and out of the gel. These hydrogels are formulated to respond to various signals such as pH [90], temperature [91], light [92], glucose [93], antigens [94], electric field [95] and magnetic field [96], to mediate changes in swelling. These stimuli-responsive polymeric hydrogels that react to changes in the environmental conditions have been extensively studied and used as smart materials for various biomedical applications.

Especially, pH-sensitive hydrogels have been extensively researched to develop controlled release formulations for oral delivery of insulin. These ionic hydrogels show sudden or gradual changes in their dynamic and equilibrium swelling behaviour as a result of changing the external pH. There are two main types of pH-sensitive hydrogels, acidic hydrogels and basic hydrogels. Acidic hydrogels get charged and swell at high pH due to ionisation and shrink at low pH [97]. While, basic hydrogels show opposite swelling behaviour in response to pH [98]. The pH sensitivity is due to the presence of acidic and basic groups such as carboxylic acids, sulphonic acids, primary amines, and quaternary ammonium salts. Acidic groups such as carboxylic acid groups are charged at high pH and uncharged at low pH, whereas the reverse is true for basic groups.

Another strategy involves the diffusion of insulin out of the gel matrix in response to the external temperature. These hydrogels help in the diffusion of dissolved insulin molecules by imbibed water out of the gel matrix while insulin crystals are retained in the matrix. This occurs only when the pore size of the matrix becomes larger than the size of single insulin molecule. External factors such as pH, temperature and thermal cycling operations, elicit the change in the pore size that controls the insulin release rate from the hydrogel, in a stimuli-response process. In one of the studies [99], the pH-and/or temperature sensitive hydrogel composed of *N*-isopropylacrylamide (NIPAAm) and *N,N'*-diethylaminopropyl methacrylamide (DMAPMAAm), exhibited a pH-sensitivity around pH 7.4 as well as a temperature-sensitivity around 37 °C. The insulin release profiles investigated under different conditions showed that temperature and thermal

cycling operations largely affected the insulin release rate while pH variation did not have significant influence on the insulin release profiles. Light also shows direct influence on the balance of attractive and repulsive forces inducing reversible shrinkage in the polymer gels. Radiation force generated by a focused laser beam induces volume phase transitions, rather than local heating, modifying the weak interactions in the gels. Due to shear-relaxation processes, gel shrinkage occurs up to several tens of micrometres away from the irradiation spot, suggesting that the combination of stimuli-responsive polymer gels and laser light may be used as an alternative to new gel-based systems for applications such as actuating or sensing [87].

Despite a promising delivery system for proteins and DNA, the responsive nature of the hydrogels is limited by diffusion of chemical signals into the gel matrix. Another disadvantage is the low water solubility of many drugs which affects the gel loading process in a drug concentrate. Furthermore, in the case of therapeutic proteins, the non-aqueous solvents used to make concentrated low-molecular-mass drug solutions often denature most bioactive proteins. Therefore, there exists the need to improve time response in order to facilitate the use of hydrogels in many practical applications including flow control elements in drug delivery systems. Therefore, hydrogels can be used as the basis of the design of closed-loop drug delivery devices for therapeutic agents used for the management of diabetes mellitus [100].

Microcapsules

In 1964, Chang [101] proposed the idea of using ultrathin polymer membrane microcapsules for the immunoprotection of transplanted cells. He coined the term 'artificial cells' to define the concept of bioencapsulation, which was successfully implemented 20 years later to immobilise xenograft islet cells. The first successful implantation of microencapsulated islets into rats by Lim and Sun, showed controlled glucose levels for several weeks [102]. The islet cells, encapsulated in calcium alginate coated with polylysine (PLL), were able to maintain normal blood sugar level in diabetic rats for 2-3 weeks. Since then, a considerable progress has been made towards understanding the biological and technological requirements for successful transplantation of encapsulated cells in experimental animal models, including rodents and non-human primates. Over the past decades, various devices for physiological controlled administration of insulin for the treatment of diabetes mellitus have been reported in literature. As mentioned earlier, an extensive amount of work has been focussed on the implantable bioartificial pancreas, producing insulin on physiological demand. These endocrine pancreatic cells are isolated, purified and enclosed in microcapsules [103] or hollow fibres [104], where a semipermeable membrane serves as a diffusion barrier. Bioencapsulation is also used for a range of therapeutic treatments for diabetes [105], haemophilia [106], cancer [107] and renal failure [108].

Owing to the very gentle, simple and rapid immobilisation procedure, an extensive volume of literature has been devoted to *in vivo* and *in vitro* applications of bioencapsulation [109, 110]. Different polymers such as alginate [111, 112], pectin [112, 113], chitosan [114], carrageenan [112] and hyaluronic acid [115] have been used for the encapsulation and delivery of a variety of proteins and cells. Generally, microbeads are prepared by extruding solutions of the polymer containing the desired protein, as droplets, into a polycation solution by the mechanism of gelation and cross-linking. The main function of the core material is to entrap the cells / protein rapidly under mild conditions with the formation of a perfect spherical bead and serve as a template for binding of the polycation; thus, porosity and surface charge density controlling the final membrane structure. On the other hand, the function of the polycation is to form a strong complex membrane to stabilise and strengthen the ionic gel network and reduce and control the permeability. Encapsulated proteins are released by two mechanisms:

(1) diffusion of the protein through the pores of the polymer network

(2) degradation of the polymer network. Bioavailability of these encapsulated drugs depends on the various characteristics of the beads such as bead morphology (shape, size, porosity / pore size) [116]; swelling power; viscosity of the polymer solution; encapsulation efficiency; pH; molecular weight of the protein [117] and charge on the protein [118, 119].

Some of the commonly used methods for microbead preparation are atomisation, emulsification and coacervation [120]. Solutions containing the polymer and protein are loaded into a syringe mounted on a syringe pump and is then delivered through an atomisation device with a defined diameter orifice at the tip. Bead size can be controlled by nitrogen gas pressure, the flow-rate of the syringe pump or the distance between the orifice and the surface of the crosslinking solution. Fine droplets of polymer and protein solution will form the microbeads when cross-linked with the polycation solution. The second method of microbead preparation involves protein encapsulation by oil-in-water emulsification technique [121-124]. This encapsulation method is suitable for stable peptides and proteins or synthetic low molecular weight drugs since it uses stronger chemicals such as ethyl ether to remove the oil at the end of the process. The size of the microbeads formed by this technique depends on the stirring speed and the rate of the addition of the cross-linking solution. In the third method, under specific conditions of polyion concentration, pH and ionic strength, the polyelectrolyte mixture separates into two distinct phases; a dense coacervate phase containing the microbeads and a dilute equilibrium phase [125].

Microencapsulation offers various benefits over other drug delivery systems in terms of controlled release of insulin, increased stability and protection of insulin from the various digestive enzymatic actions in the GI tract and enzymatic proteolysis and acidic degradation of orally administered insulin in the stomach, using a biodegradable polymeric material which makes it a suitable system for pulmonary delivery. In addition, the controlled release preparation of insulin can continuously exhibit pharmaceutical efficacy *in vivo* in a stable manner for an extended period of time. Drug bioavailability can be further enhanced by conjugating it to molecules that can recognise specific receptors on the epithelial cells and get transported across the intestinal epithelium.

Along with increased efficacy, this approach eliminates the side effects associated with prolonged opening of the cross-linked junctions. This also avoids the unspecific transport of toxic compounds due to the passage across cell barrier by the transcellular mechanism and specificity towards the protein of interest.

We combined the concept of synthetic cells and microencapsulation by encapsulating mouse embryonic stem cells in alginate matrix; which on differentiation formed pancreatic insulin-producing cells *in vitro*; thus providing alternative cell sources for the treatment of *type 1* diabetes for the closed loop delivery of insulin. To achieve this goal, the differentiation strategy from mouse ES cells towards insulin-producing cells was designed and optimised to obtain relatively large amount of insulin-producing cells with fully characterised pancreatic functionalities in order to fulfil the requirements for cells transplantation strategies in the treatment of *type 1* diabetes. Mouse embryonic stem (ES) cell line, CEE14, was used and optimised culture conditions were determined such as the cells culture surfaces, cells original seeding densities and cells differentiation characters. The differentiation protocol was optimised in terms of time, lineage selection and new growth factors. The encapsulating agent, alginate matrix was characterised for the activity of the insulin producing stem cells on the basis of maintenance and proliferation of mouse ES cells within alginate micro-beads and differentiation of mouse ES cells within alginate matrix. Different alginate compositions (0.5 %, 1.0 % and 2.0 % w/v) were examined for the encapsulation of the stem cells. Finally cells differentiation towards pancreatic insulin-producing cells was evaluated in comparison with two dimensional culture system.

It was reported that with currently used encapsulation technique, ES cells containing alginate microbeads can be maintained in the culture medium for relatively long period of time (at least for 30 days) without detectable morphology changes (unpublished data). Scanning electron microscope (SEM) images (Fig. **3**) clearly showed the porous structure within alginate matrix; the average pore size for 0.5 % w/v alginate beads was 10.9 ± 1.0 µm, for 1 % w/v the size decreased to 5.0 ± 0.5 µm and for 2% w/v the size was only 3.4 ± 0.77 µm inferring that the pore size is dependent on the initial concentration of the alginate used in the preparation.

It indicated that 1.0 % w/v alginate matrix proved to be the best encapsulating composition for supporting and protecting the encapsulated cells in the *in vitro* culture system. It should be noted that 0.5% w/v alginate matrix formed a bead with a relatively 'weak' structure, prone to collapse and lacking an ability to maintain the morphology, hence resulting in the release of encapsulated cells.

Differentiated ES cells were also challenged by low (5 mM) and high (20 mM) level of glucose to study their insulin release pattern. It clearly demonstrated that differentiated cells within alginate beads responded positively to the glucose stimulation, and insulin released into the culture medium in a glucose dose dependent manner; 5mM glucose challenging, 3D differentiated ES cells released 63.62 ± 15.09 ng/mg protein insulin, and under 20 mM glucose challenging, the insulin amount increased to 127.64 ± 12.22 ng/mg protein (Figs. **5**, **6**).

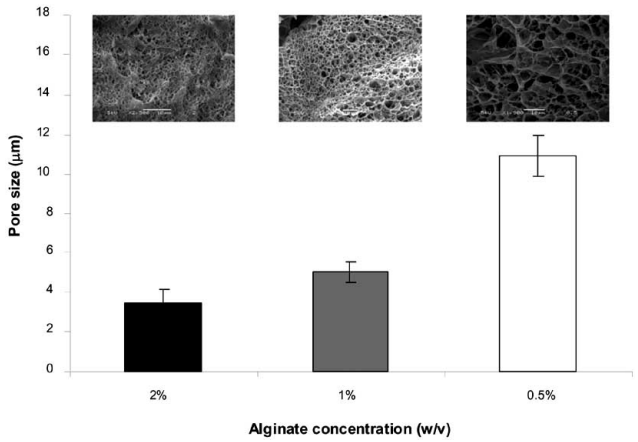

Fig. (3). Measurement of alginate beads intracapsular pore size at various alginate concentrations with the Cryo-SEM images inset.

The insulin production under glucose increased about 1.5 times and 1.8 times under low level and high level glucose stimulation. After several optimisations, the best insulin yield within two dimensional differentiation was 42.49 ± 0.86 ng/mg protein and 72.24 ± 2.45 ng/mg protein under 5mM and 20mM glucose challenging respectively. Thus, three dimensional differentiations seemed to offer better insulin productivity compare with conventional two dimensional differentiation.

Through this study, we tried to introduce a 3D alginate-based culture system for the enhanced maintenance and differentiation of mouse embryonic stem cells. This system uses calcium based alginate microbeads which is biodegradable and highly hydrated to allow cells attachment. The study clearly demonstrated that the alginate micro-beads can be produced with optimised properties to meet essential cells culture requirements. This system offered many advantages such as it can be used to resist the immuno-rejection membrane in addition to its ability to build 3D micro environment to enhance cells differentiation. This study represented the additional advantage of alginate to be used as encapsulation material. By simply varying the alginate concentrations, different molecular weight molecules diffusion can be controlled which may prove to be beneficial for therapeutic bio-efficient and bio-effective drug delivery. Therefore, we conclude that alginate encapsulate micro-beads provide a scalable system to control mouse ES cells differentiation into pancreatic insulin-producing cells.

Despite having significant achievements in controlling the blood glucose levels, there are some disadvantages associated with the drug delivery system. Some of the primary disadvantages are lack of reproducibility, lack of standardised technology and production of uniform capsules. However, this can be overcome by making the process automated. Another disadvantage is the lack of clinical-grade polymers. Although alginate has been the most effective encapsulation material of choice, batches of alginate need to be standardised to minimise endotoxin and protein content as this may

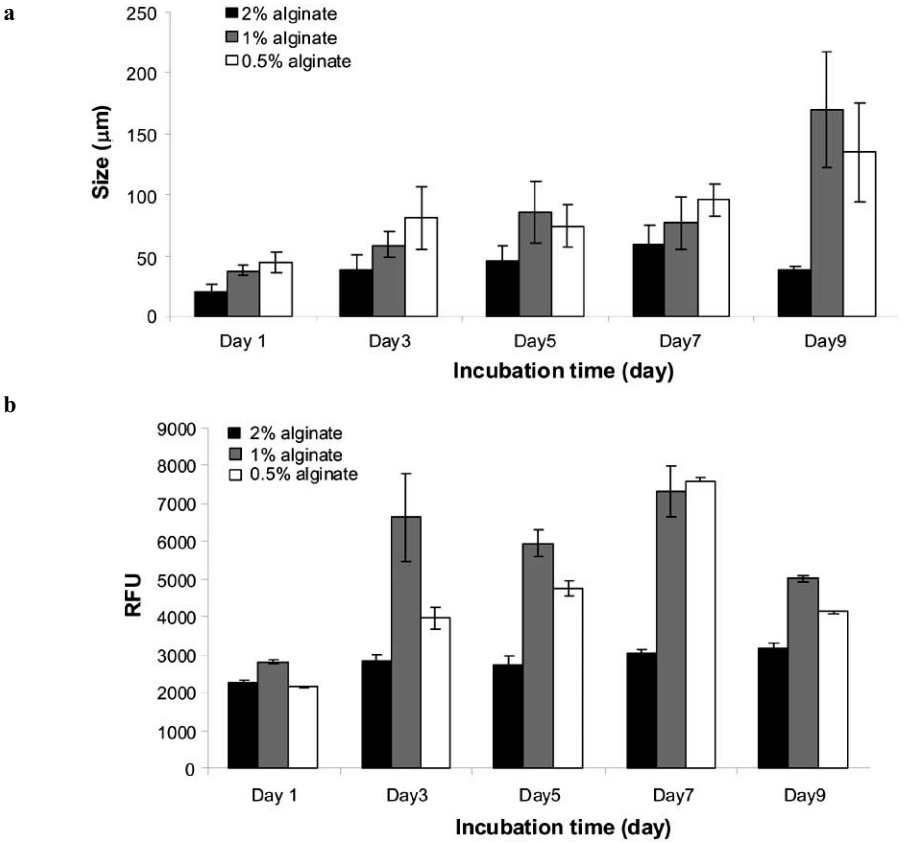

Fig. (4). ES cells proliferation study within various concentrations of alginate beads. (**a**) ES cells proliferation within various concentrations alginate, assessing by diameter measurement of ES cells clusters; (**b**) ES cells proliferation within various concentrations alginate, assessing by Alamar blue assay.

affect biocompatibility of the system. Other factors affecting the successful application of the system include suitable immune-compatible polycations [126], assessment of the exact dosage [127] and molecular-weight cut-off value, suitable cell types for immobilisation, appropriate source of functional cells, choice of transplantation site and regulatory and ethical issues [128].

CURRENT AND FUTURE DEVELOPMENTS

Several attempts have been made to adopt various effective measures for the surveillance, prevention and control of diabetes and its complications, particularly in low and middle-income countries. In the attempt to make the diabetes treatment more acceptable, many alternative delivery sys-

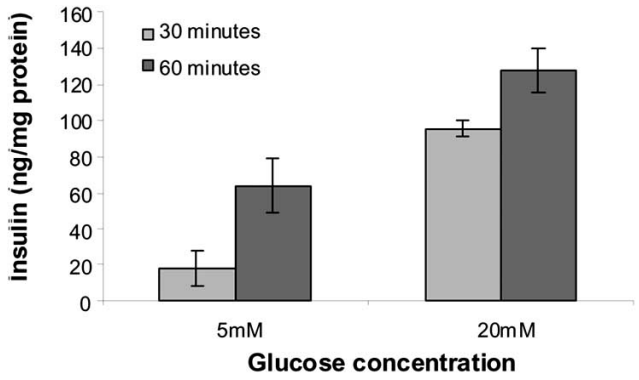

Fig. (5). Insulin production by three dimensional differentiated ES cells after glucose challenging. ES cells containing alginate beads were exposed into glucose solutions for 30 minutes and 60 minutes in order to study the insulin retention within alginate capsules.

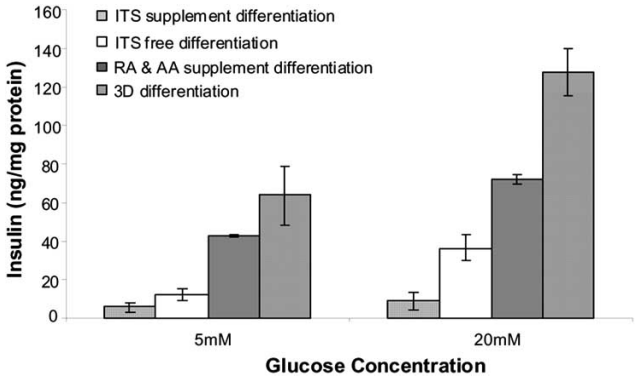

Fig. (6). Comparison between insulin productions between two dimensional differentiated ES cells and three dimensional differentiated ES cells after glucose challenging. RA: ITS: insulin-transfferin- selenium.

tems and routes of insulin administration have been explored and many challenges are undertaken. Insulin delivery by alternative route is the area of current interest in the design of drug delivery system. In the endeavour to develop alternative insulin delivery technologies, most of the global pharmaceutical companies are showing encouraging progress. It appears that years of persistent research into non-injectable methods of insulin delivery have resulted in some clinically available routes of insulin administration. Among the various routes of insulin administration pulmonary route appears to be the most clinically viable non-invasive system with the advent of new delivery devices such as dry powder and liquid aerosol formulations. This route of administration has proved to be as effective and well tolerated as the subcutaneously injected regular insulin, and also has a pharmacodynamic profile better suited for mealtime insulin therapy. Other methods of insulin delivery (especially oral) may prone to be a potential choice for prandial replacement. Although the absorption efficiency across epithelial surfaces of the mouth, gastrointestinal tract, skin and nose has increased by using absorption-enhancing techniques, bioavailability of drugs through these routes still has low clinical value. Synthetic beta cells offer advantage in insulin secretion in terms of their response to blood glucose levels in the microenvironment as compared to the exogenous insulin administration. However, various limitations such as inefficient production of functional insulin-secreting cells from pluripotent progenitors and immune response have decreased their popularity as a novel insulin delivery system. Various delivery systems such as hydrogels and microcapsules have proved to be effective as closed loop delivery systems for insulin. Despite these promising developments, these strategies need to be evaluated completely as potential therapeutic alternatives to overcome the limitations such as precision of dosing, dose adjustments and reproducibility.

In a nut shell, the ultimate goal for the treatment of diabetes remains the development of a fully automated glucose-controlling device. Although the search for alternative routes to subcutaneous insulin administration has been relatively unsuccessful, recent approaches seem to hold potential for effective insulin therapy. Though each system has its own set of favourable and unfavourable properties, yet these unfavourable aspects need to be circumvented to ease the burden of traditional insulin regimens, making them potential alternative insulin delivery systems and reach the market. In addition methods of bioavailability estimation also need to be standardised for meaningful comparisons among different delivery devices. Some other factors such as cost, ease of administration may also determine their use as successful insulin delivery systems to the ever-increasing diabetic population. These delivery systems seem to bring a revolutionary change in the delivery of insulin in the near future, for the millions of diabetic patients relying on subcutaneous administration.

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