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Strategies for improving diabetic therapy *via* alternative administration routes that involve stimuli-responsive insulin-delivering systems

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ABSTRACT

The encapsulation of insulin in micro- or nanodelivery systems may eliminate the need for frequent subcutaneous injections, improving the quality of life of diabetic patients. Formulations for oral, intranasal, pulmonary, subcutaneous, and transdermal administration have been developed. The use of stimuli-responsive polymeric carriers that can release the encapsulated drug in response to changes of the environmental stimuli or external activation enables the design of less invasive or non-invasive systems for smart insulin delivery from depots in the body. This article will look at strategies for the development of responsive delivery systems and the future meeting of the demands of new modes of insulin delivery.

Contents

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1. Introduction

Diabetes mellitus is an endocrine disorder that is characterized by high blood glucose levels (hyperglycemia), ultimately leading to numerous serious and even fatal complications. It is caused by the production of either too little or no insulin as a result of destruction of pancreatic β-cells (type 1 diabetes) or reduced insulin sensitivity, which is followed by insufficient insulin secretion because of progressive β-cell exhaustion (type 2 diabetes) [1–3]. The current standards of care for patients with either type 1 or 2 diabetes involve the administration of insulin, a 51-amino-acid peptide [4]. In response to hyperglycemia, insulin regulates blood glucose levels by stimulating cells in the body, specifically liver and skeletal muscle cells, to take up glucose from the blood [5,6].

The conventional approach of controlling hyperglycemia for the treatment of diabetes involves multiple subcutaneous injections of exogenous insulin [7–9]. Although without acute complications, daily subcutaneous injections can cause discomfort, infection, and hyperinsulinemia, resulting in poor patient compliance [8]. Moreover, such injection therapy commonly leads to the suboptimal control of blood glucose levels because glucose sensing and insulin delivery are not directly coupled [10].

To overcome the psychological stress and shortcomings that are associated with the above conventional treatment, efforts have been made to identify alternative, less invasive or non-invasive routes particularly those involving stimuli-responsive systems for insulin delivery. Strategies that employ stimuli-responsive insulin-delivering carriers or devices to improve diabetic therapeutic delivery are rapidly becoming more mature. This review will elucidate alternative routes for insulin delivery (including oral, intranasal, pulmonary, subcutaneous, and transdermal administrations) (Fig. 1) that use various environmentally or externally regulated stimuli-responsive micro- and nanocarrier systems. The encapsulation of insulin in these stimuli-responsive vehicles may eliminate the need for frequent subcutaneous injections, improving patient safety and compliance. The challenges in meeting the demands of insulin delivery *via* alternative administration routes will also be discussed. Since the subject of transdermal insulin delivery will be covered in another review, it is not discussed herein.

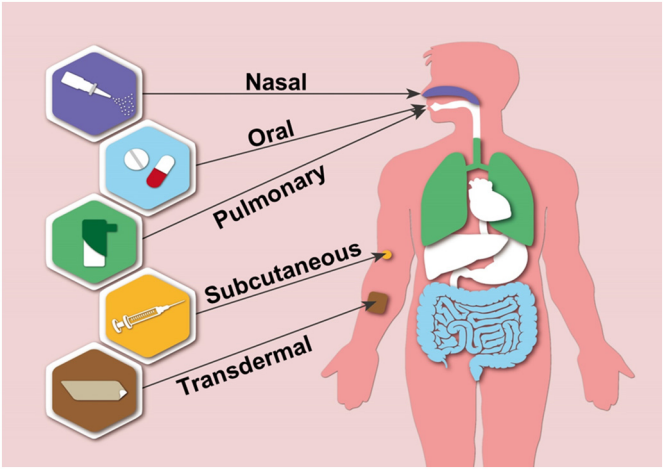

Fig. 1. Alternative administration routes for insulin for treating diabetes, including nasal, oral, pulmonary, subcutaneous, and transdermal. Adapted with permission from [59].

2. Oral delivery of insulin

Oral administration of therapeutic agents remains the most patient-friendly route, as it is non-invasive and convenient. However, the oral delivery of peptide/protein drugs such as insulin is highly challenging, as these hydrophilic macromolecules have to overcome several major challenges in the gastrointestinal (GI) tract, such as low pH and protease hydrolysis [11,12]. Additionally, owing to their high molecular weights and hydrophilicity, the permeability of peptide/protein drugs across the intestinal epithelium that is covered by mucous layer is rather poor [13]. A carrier system is therefore required to protect its loaded therapeutic agents against the harsh GI environment and facilitate their crossing the epithelial barrier and being transported into systemic circulation.

The intestinal epithelium comprises a cell layer of enterocytes, goblet cells, and Microfold (M) cells [14]. The absorption of therapeutic agents across the epithelial barrier can occur by either transcellular or paracellular diffusion [15]. However, hydrophilic peptide/protein drugs cannot directly diffuse through the hydrophobic lipid-bilayer membranes of epithelial cells, and the transport of therapeutics *via* the paracellular pathway is severely restricted by the presence of tight junctions at the luminal aspect of adjacent epithelial cells [16,17]. Numerous intestinal permeation enhancers that promote drug absorption *via* the transcellular and/or paracellular pathways have been studied [18–20]. Many stimuli-responsive polymeric carriers that involve these permeation enhancers that can control oral insulin delivery (depending on the environmental pH, glucose concentration, or external stimuli) and enhance its bioavailability have been reported.

2.1. pH-responsive carriers

The pH values in the GI tract rise rapidly from highly acidic (pH 1.0–3.0) in the stomach to pH 6.0–6.5 in the duodenum, and increase further to neutral or slightly alkaline along the jejunum and ileum (pH 7.0–7.5) [21]. Various microparticle (MP) and nanoparticle (NP) carrier systems, which function as smart on–off switches for the controlled release of insulin in a manner related to the pH in the GI tract, have been investigated [22–24]. These pH-responsive carriers are stable and can protect encapsulated therapeutic proteins under acidic conditions in the gastric environment, while allowing the controlled release of the cargoes at neutral pH when they reach the small intestine.

The pH-responsiveness of a carrier system is usually caused by weakly acidic or basic functional groups on the backbones of its constituent polymeric materials, such as polyacrylic acid (PAA) and chitosan (CS), whose electrostatic charges can be tuned in response to pH changes in the local milieu [25]. PAA, a biocompatible polymer that contains carboxylic acid groups can bind with Ca^{2+} ions that are present in the intestinal fluid and mucus layer [26]. Insulin is very sensitive to trypsin and chymotrypsin, which are Ca^{2+}-dependent enzymes in the GI tract [27]. One method for inhibiting the activities of intestinal proteolytic enzymes is to remove Ca^{2+} ions from the enzyme structures [28]. The removal of extracellular Ca^{2+} ions may also disrupt intercellular tight junctions, increasing the paracellular permeability [20,29]. CS is another polymer of natural-origin that has been widely used as an excipient in oral drug delivery [14,18]. CS is a well-known mucoadhesive agent that has been used as a paracellular permeation enhancer that can reversibly open epithelial tight junctions to increase the intestinal absorption of therapeutic macromolecules [30–32].

Gao and coworkers synthesized a PAA-based hydrogel as a pH-responsive carrier system for oral insulin delivery [24]. The p*K*a value of the carboxylic acid groups on PAA is approximately 4.5 [33]. Insulin molecules can be protected in this carrier system in the acidic environment of stomach. The carboxylic acid groups in PAA are deprotonated at the neutral pH of the small intestine, generating electrostatic repulsion between the polymer chains, allowing the encapsulated drug to be released (Fig. 2A). Lyophilized insulin-loaded gels were ground into MPs with a size of 100–200 μm and then orally administered to the rats with streptozotocin (STZ)-induced diabetes using an oral gavage needle. A continuous decline in blood glucose levels was observed, which was maximized at 6 h post-administration, and then gradually returning to the initial level (Fig. 2B).

Sonaje et al. prepared an NP delivery system that comprised the positively charged CS and the negatively charged γ-poly-glutamic acid (γ-PGA) for oral insulin delivery [22]. The as-prepared NPs were pH-responsive as the p*K*a of CS is around 6.5 [34]. In an environment with a pH of <7.0 such as the lumen of the duodenum, the CS NPs remained intact, but at pH > 7.0, as in the mucus layer close to the intercellular spaces, they became unstable and disintegrated (Fig. 2C). The mechanism of the oral insulin delivery is as follows. The mucoadhesive CS NPs adhere to and infiltrate the mucus layer that covers the small intestine. The infiltrating NPs then transiently open the tight junctions between epithelial cells, while becoming unstable and disintegrating, as a result of their pH-responsiveness, releasing the loaded insulin. The released insulin then permeates the opened paracellular pathway and is absorbed into systemic circulation, exerting glucose-lowering effects.

In addition to the above-mentioned examples, derivatives of PAA (poly(methacrylic acid)) [35–38] and CS (*N*-trimethyl CS and lauryl succinyl CS) [23,39,40] as well as their polyelectrolyte complexes such as PAA/CS [41–43], PAA/liposome [44], CS/γPGA [22,45–47], and CS/alginate [48–50] have been proposed as pH-responsive carrier materials for oral insulin delivery. Table 1 presents representative examples of pH-responsive micro- and nanocarriers that have been recently found to improve oral bioavailability of insulin [51–56].

Although research into oral insulin delivery has mostly focused on the use of pH-responsive carriers, other appealing methods that use magnetically responsive or glucose-responsive carriers have been proposed for prolonging intestinal retention of delivery vehicles and triggering drug release, respectively.

2.2. Magnetically responsive carriers

Langer and coworkers reported a formulation strategy that uses an external magnetic field to localize and prolong the residence time of poly(lactide-*co*-glycolide) (PLGA) MPs that contain magnetite crystals and insulin in the intestinal area (Table 1) [57,58]. The hypoglycemic effects of the magnetically responsive carriers were evaluated in a mouse

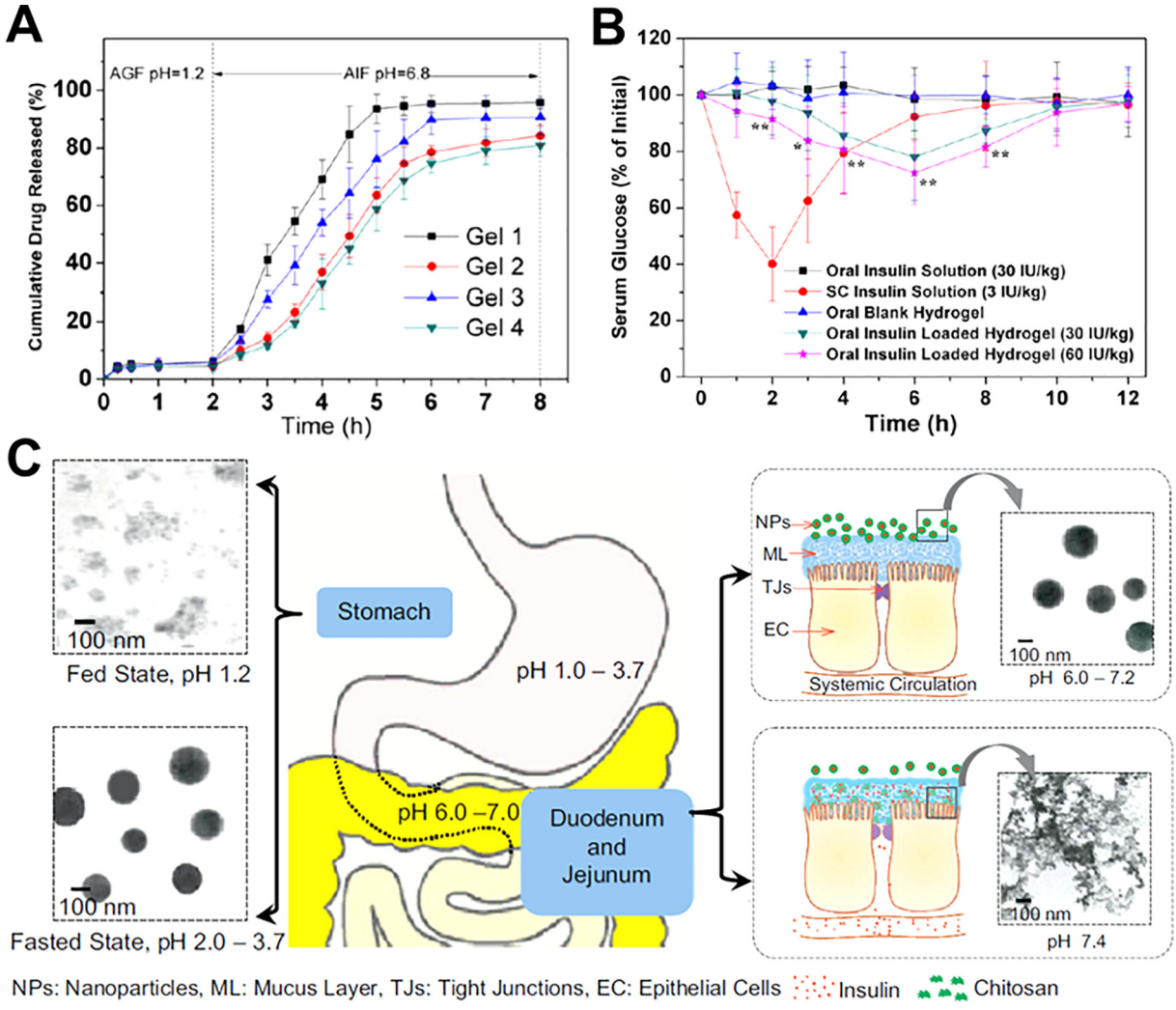

Fig. 2. pH-responsive insulin delivery *via* oral administration. (A) Release profiles of insulin from PAA-based hydrogels following incubation in artificial gastric fluid (AGF) for 2 h, followed by incubation in artificial intestinal fluid (AIF) for another 6 h. (B) Blood glucose levels of diabetic rats following various treatments. (C) Presumed mechanism of paracellular transport of insulin released from pH-responsive CS NPs. NPs: nanoparticles; ML: mucus layer; TJs: tight junctions; EC: epithelial cells. Adapted with permission from [22,24].

Table 1.
Selected examples of stimuli-responsive insulin delivery by oral administration.

Triggering stimuli	Materials	Carrier formulations	Triggering methods	Insulin doses	Animal model	References
pH	PAA-based	PAA/CMC	Intestinal pH	30, 60 IU/kg	Wistar rat	[24]
		PAA/PEG	Intestinal pH	50 IU/kg	Wistar rat	[35]
		PAA/PGA	Intestinal pH	30, 60 IU/kg	Wistar rat	[36]
		PAA/*O*-carboxymethyl CS	Intestinal pH	40 IU/kg	Sprague-Dawley rat	[41]
		PAA/*N*-succinyl CS	Intestinal pH	50, 100 IU/kg	Swiss albino mouse	[42]
		PMAA/PEG	Intestinal pH	25 IU/kg	Sprague-Dawley/Wistar rat	[37,38]
		PMAA/carboxylated-CS	Intestinal pH	25, 50 IU/kg	Sprague-Dawley rat	[43]
	CS-based	CS/γPGA	Intestinal pH	30 IU/kg	Wistar rat	[22,45–47]
		CS/alginate	Intestinal pH	50, 100 IU/kg	Swiss albino mouse/Sprague-Dawley/Wistar rat	[48–50]
		CS/TPP	Intestinal pH	50–100 IU/kg	Sprague-Dawley/Wistar rat	[51,52]
		Ttrimethyl CS/Cysteine	Intestinal pH	50 IU/kg	Sprague-Dawley rat	[39]
		Trimethyl CS/CSK peptide	Intestinal pH	50 IU/kg	Sprague-Dawley rat	[23]
		Lauryl succinyl CS	Intestinal pH	60 IU/kg	Wistar rat	[40]
	PLGA-based	PLGA NPs	Intestinal pH	76.6 IU/kg	Sprague-Dawley rat	[53]
		PLGA NP encapsulated into microcapsules	Intestinal pH	20 IU/kg	Wistar rat	[54,55]
	Lipid-based	FA-PAA-PAH-modified liposomes	Intestinal pH	50 IU/kg	Sprague-Dawley rat	[44]
		TP/SC/PLL	Intestinal pH	25 IU/kg	Sprague-Dawley rat	[56]
Magnetic field	–	Magnetic NPs in PLGA MPs	Magnets (1 × 1 × 0.5 in.) applied to abdominal area of mice	100 IU/kg	Balb/c mouse	[57]
	–	PLGA MPs-micromagnet complexes	Magnetic belt applied to abdominal area of mice	120 IU/kg	Balb/c mouse	[58]
Glucose	–	FA-PBA-PAA NPs in HA hydrogel	Glucose levels	75 IU/kg	Sprague-Dawley rat	[60]

Abbreviations: PAA, polyacrylic acid; CS, chitosan; PLGA, poly(lactic-co-glycolic acid); CMC, carboxymethyl cellulose; PEG, polyethylene glycol; PGA, polyglutamic acid; PMAA, poly(methacrylic acid); TPP, tripolyphosphate; CSK, CSKSSDYQC; NPs, nanoparticles; FA, folic acid; PAH, poly(allyl amine) hydrochloride; TP/SC, *N*-tocopheryl-*N*′-succinyl; PLL, poly-L-lysine; MPs, microparticles; PBA, phenylboronic acid; HA, hyaluronic acid.

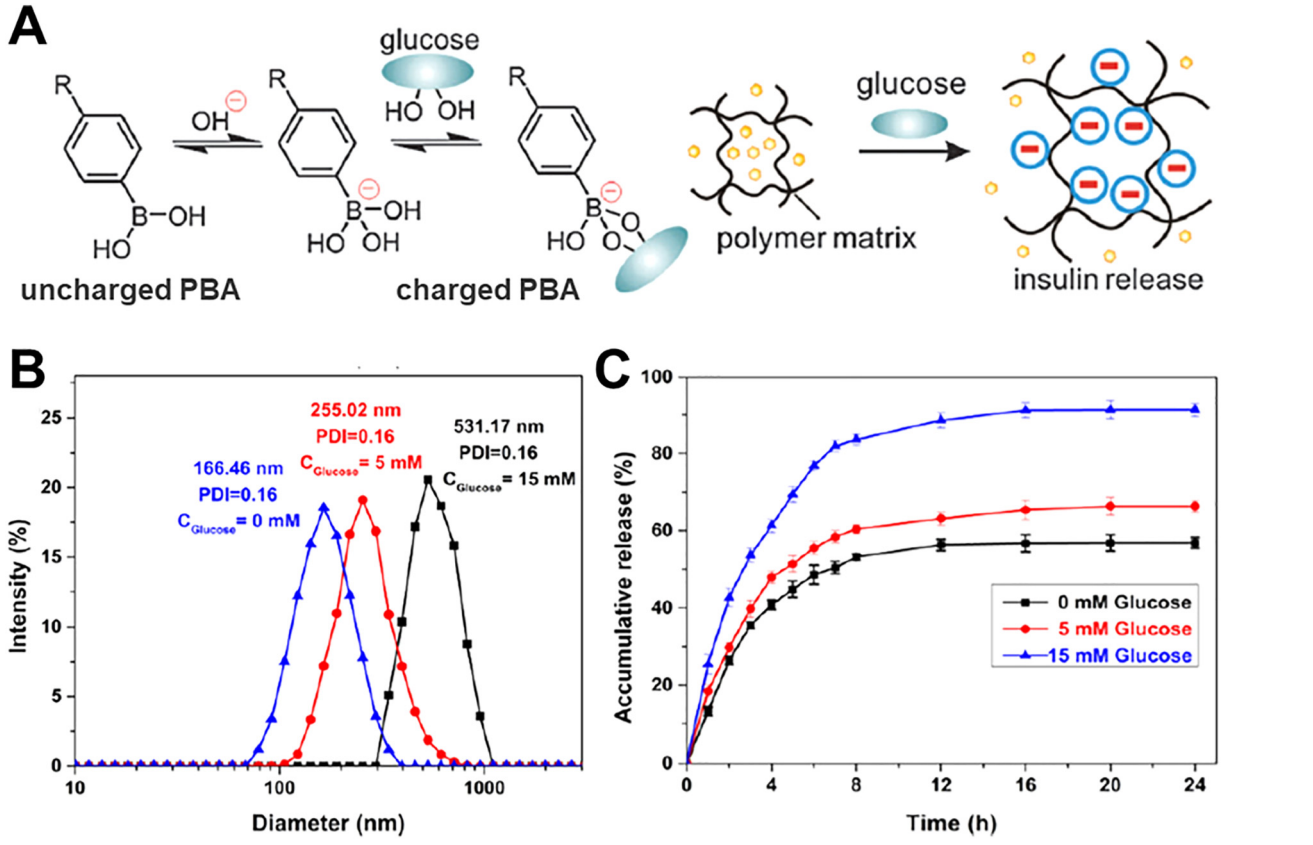

Fig. 3. Glucose-responsive insulin delivery *via* oral administration. (A) Mechanisms of insulin release from PBA-based glucose-responsive system. (B) Results of dynamic light scattering analysis. (C) Insulin release profiles of PBA-based glucose-responsive NPs in various concentrations of glucose. Adapted with permission from [59,60].

Table 2
Selected examples of stimuli-responsive insulin delivery by intranasal administration.

Triggering stimuli	Carrier formulations	Triggering methods	Insulin doses	Animal model	References
Temperature	Poloxamer/CS/glycine/glutaraldehyde	37 °C, sol-gel transition (solution transformed into hydrogel, decreasing nasal mucociliary clearance rate)	10 IU/kg	Wistar rat	[67]
	HTCC/PEG/α,β-glycerophosphate	37 °C, sol-gel transition	10 IU/kg	Sprague-Dawley rat	[68]
	Trimethyl CS/PEG/α,β-glycerophosphate	37 °C, sol-gel transition	10 IU/kg	Sprague-Dawley rat	[69]
pH	Carbopol/HPMC	pH 7.2–7.4	1.5 IU/kg	Sprague-Dawley rat	[70]

Abbreviations: CS, chitosan; HTCC, *N*-((2-hydroxy-3-trimethylammonium) propyl)) chitosan chloride; PEG, polyethylene glycol; HPMC, hydroxypropyl methylcellulose.

model in the presence of a circumferentially applied external magnetic field. The results thus obtained revealed that prolonging the intestinal residence time of insulin delivery vehicles improves blood-glucose-lowering as more insulin is released and then absorbed.

2.3. Glucose-responsive carriers

Polymers that are functionalized with phenylboronic acid (PBA) have been extensively studied as glucose-responsive carrier materials for insulin delivery, owing to the highly specific interaction between the boronic acids on PBA and the 1, 2-diols in glucose (Fig. 3A) [59]. A glucose-responsive NP system that comprises PBA functionalized polymers has been proposed as an alternative system for administrating insulin *via* the oral route (Table 1) [60]. To target the folate receptors that are overexpressed on the intestinal epithelial cells, folic acid is introduced onto the NPs, facilitating the delivery of NPs by receptor-mediated endocytosis. The as-prepared NPs are significantly enlarged in the presence of glucose (Fig. 3B), allowing insulin release (Fig. 3C). The oral administration of the insulin-loaded NPs in diabetic rats had an effective hypoglycemic effect, with the greatest depression of the blood glucose level 5 h later.

Although treatment with insulin *via* the oral route is highly challenging, oral insulin takes advantage of its direct absorption into the liver *via* portal circulation, which is a route that replicates the endogenous insulin secretion [61]. The direct absorption of insulin into the liver, as its primary action site, has been regarded as being physiologically beneficial, although its long-term effects are yet to be elucidated. Conversely, the subcutaneous administration of insulin targets peripheral tissue rather than the liver, and so is dissimilar to the physiological route of insulin.

3. Intranasal delivery of insulin

The nasal cavity has attracted much interest as an accessible location for non-invasive drug delivery. Drugs that are delivered *via* the intranasal route using a nasal metered spray or a nasal pipette can be rapidly transported into the systemic circulation, owing to the large surface area (*ca.* 150 cm^2) and high vascularity of the nasal mucosa for drug absorption [62–64]. The mechanism of the intranasal delivery of insulin involves the adhesion of the drug carriers to the mucous layer and the passage of the drug through the nasal epithelium, followed by its direct transport into systemic circulation, bypassing the hepatic first-pass metabolism [11,59,62,65]. In spite of the effectiveness of the intranasal route, the nasal delivery of insulin presents fundamental challenges, including the short residence time of a liquid dosage and the barrier of the nasal mucosa to the absorption of the drug, limiting its bioavailability [11,62,66].

Methods for using *in situ* gelling polymers such as Poloxamer, CS, and Carbopol that can prolong the residence time of drug carriers in the nasal cavity and increase drug permeability across the nasal mucosa have been examined for the purpose of insulin delivery [67–70]. *In situ* gelling polymers can undergo a sol-gel transition in aqueous solutions, induced either by environmental temperature or pH upon intranasal administration [71,72]. Table 2 presents the formulations, gelation-triggered mechanisms, effective doses of the therapeutics, and the animal models.

3.1. Temperature-responsive systems

Poloxamers are nonionic triblock copolymers of poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide), the most commonly used thermo-responsive polymers [73], which have been formulated as *in situ* nasal gels in combination with various mucoadhesive agents [72]. The underlying mechanism of Poloxamer gelation involves its micellization and micellar aggregation in a concentration- and temperature-dependent manner [72]. Chung et al. prepared an *in situ* gelling polymer of Poloxamer 407/CS that contained insulin [67]. The duration of the *in vitro* release of insulin from this *in situ* forming gel was six times that from its counterpart with plain Poloxamer 407. The nasal administration of Poloxamer 407/CS gel to rats with experimentally induced diabetes resulted in a prolonged hyperglycemic effect.

In the presence of β-glycerophosphate (β-GP), quaternized CS derivatives such as *N*-((2-hydroxy-3-trimethylammonium) propyl) CS chloride (HTCC) and trimethyl chitosan (TMC) can rapidly gel *in situ* in response to an increase in temperature [68,69,71]. The formulated HTCC/β-GP and TMC/β-GP gels have been shown to influence the controlled delivery of insulin as governed by the environmental temperature. In animal experiments, the administration of insulin-containing hydrogel formulations *via* the intranasal route reduced blood glucose levels for *ca.* 24 h in diabetic rats, with no apparent cytotoxicity.

3.2. pH-responsive systems

Another type of *in situ* gelling polymer whose effectiveness in insulin delivery through the nasal cavity has been evaluated is Carbopol, which is a crosslinked PAA polymer that exhibits a sol-gel phase transition when induced by an appropriate local pH [71]. Insulin that is delivered

Table 3
Selected examples of stimuli-responsive insulin delivery by pulmonary administration.

Triggering stimuli	Carrier formulations	Triggering methods	Insulin doses	Animal model	References
Glucose	Con A-linked agglomerates	Glucose levels	1.4 IU/kg	Sprague-Dawley rat	[81]
	PBA-contained polymeric micelles	Glucose levels	–	–	[82]
Cysteine	DTSSP-linked agglomerates	External application of cysteine	1.42 IU/kg	Sprague-Dawley rat	[83]

Abbreviations: Con, concanavalin A; PBA, phenylboronic acid; DTSSP, dithiobis(succinimidyl propionate).

Table 4
Selected examples of stimuli-responsive insulin delivery by subcutaneous administration.

Triggering stimuli	Carrier formulations	Triggering methods	Insulin doses	Animal model	References
Glucose	GOx/CAT encapsulated in pH-sensitive materials	Glucose levels	40–60 mg/kg	C57B6 mouse	[86–88]
	SVs coated with GOx/CAT/PEI	Glucose levels	40 mg/kg	C57B6 mouse	[89]
	PBA-based polymeric nanogels	Glucose levels	4 IU/kg	Wistar rat	[90]
	PBA-modified PLGA MPs coated with hydrogel	Glucose levels	50 mg/kg	ICR mouse	[91]
	PLGA MPs coated with PBA-containing polymers	Glucose levels	1.5 mg/kg	Kunming mouse	[92]
pH/Temperature	PAE-PCL-PEG-PCL-PAE hydrogels	pH 7.4; 37 °C. sol-gel transition and degradation	1 mg/rat	Sprague-Dawley rat	[93]
Ultrasound	PLGA nano-network	Focused ultrasound system (950 kHz; 20 μs; 30 s)	60 mg/kg	C57B6 mouse	[94]
	PLGA nanocapsules in CS microgels	Focused ultrasound system (950 kHz; 20 μs; 30 s)	1.4 IU/kg	C57B6 mouse	[95]
Magnetic field	EVA copolymer mixtures	Magnetic field (2700G; 13.3 Hz)	N/A	Sprague-Dawley rat	[96]
	Magnetic NPs in Alginate/CS matrix	Magnetic field (1800G; 33 Hz)	N/A	Swiss mouse	[97]
Near infrared	Insulin reservoir capped by ethylcellulose membrane containg Au NPs	808 nm laser light (570 mW/cm^2; 30 min)	1 U/rat	Sprague-Dawley rat	[98]

Abbreviations: GOx, glucose oxidase; CAT, catalase; SVs, silica vehicles; PEI, polyethylenimine; PBA, phenylboronic acid; PLGA, poly(lactic-*co*-glycolic acid); MPs, microparticles; PAE, poly(β-amino ester); PCL, poly(caprolactone); PEG, polyethylene glycol; EVA, ethylene-vinyl acetate; CS, chitosan; Au NPs, gold nanoparticles; N/A, not available.

by the Carbopol-formulated nasal gel is absorbed quickly after application. The hypoglycemic effect of this *in situ* forming gel reportedly lasts longer in rats than in human volunteers [70].

Since the human nasal cavity can retain a limited volume of fluid (approximately 200 μL per nostril), only a small amount of the formulation can be administered intranasally [62]. The administering of excess volume of fluid into the nasal cavity would disturb the normal function of the nose. Nasal drug delivery is, therefore, primarily suitable for concentrated solutions or potent drugs.

4. Pulmonary delivery of insulin

Insulin delivery *via* the pulmonary route has been regarded as a potential alternative method to subcutaneous injection therapy for

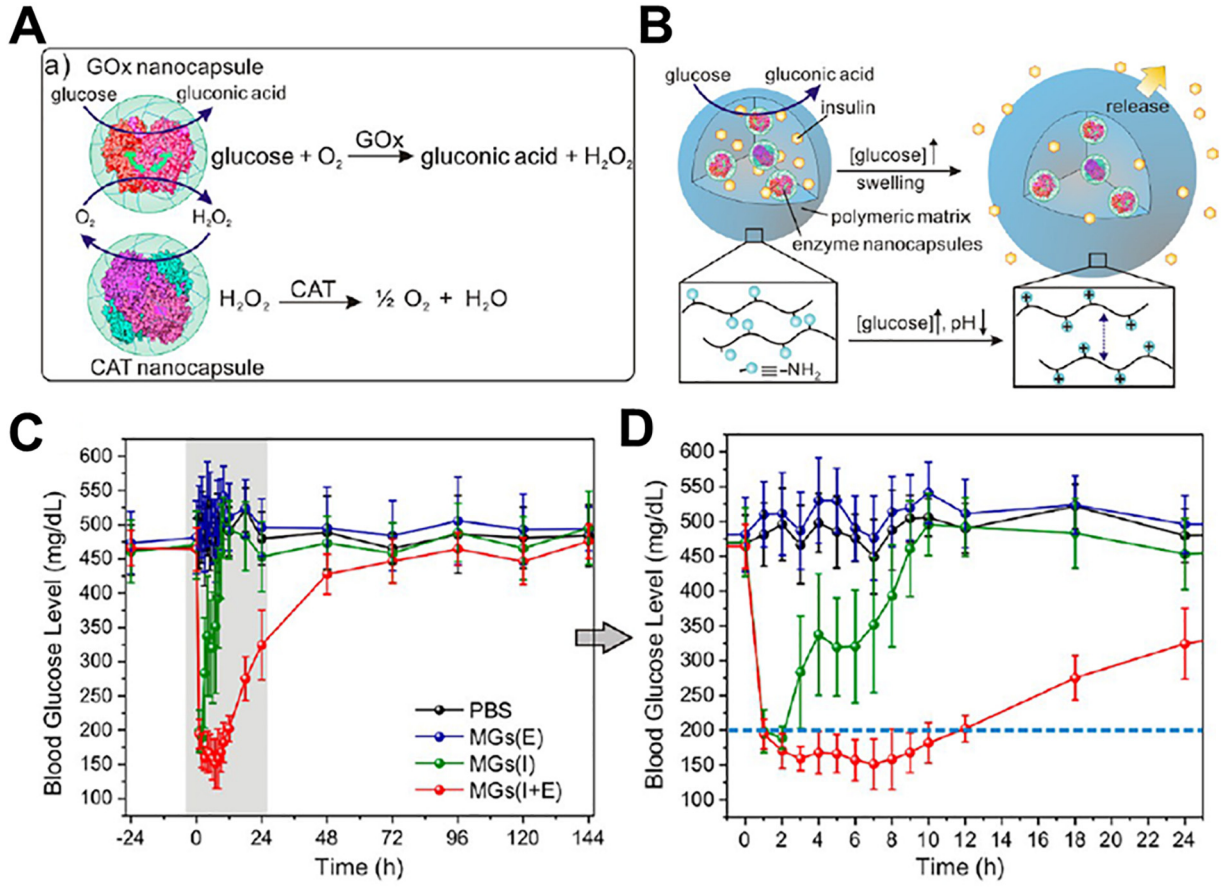

Fig. 4. Glucose-responsive carrier *via* subcutaneous administration. (A) Mechanism of enzyme-based glucose-responsive NPs. (B) Working mechanism of test MPs that encapsulate insulin and enzyme NPs as glucose-responsive insulin delivery systems. (C) Blood glucose levels of diabetic mice after subcutaneous injection with PBS, MPs that encapsulate insulin and enzymes (MGs(I + E)), MPs that encapsulate insulin only (MGs(I)) or MPs that encapsulate enzymes only (MGs(E)). (D) Blood glucose levels extracted from shaded part of (C). GOx: glucose oxidase; CAT: catalase. Reprinted with permission from [87].

treating diabetes. Pulmonary drug delivery by inhalation from the trachea through the alveolar epithelium of the lung and ultimately to the systemic circulation, can be conveniently realized using a portable inhaler or a nebulizer [74]. The alveolar epithelium is permeable to lipophilic and hydrophilic drugs, which passively diffuse *via* the transcellular and paracellular pathways, respectively [1]. The enormous absorptive surface area (80–120 m^2) and thin (0.2 μm) and highly vascularized epithelium of the alveoli can provide a high bioavailability of the delivered drug and a rapid onset of pharmacological action [75,76]. Moreover, drug delivery through the lung has the advantages of low first-pass metabolism and is relatively resistant to most peptidases, making pulmonary inhalation a favorable non-invasive route for the administration of peptide/protein drugs [77,78]. However, the rapid removal of carrier particles by mucociliary clearance from ciliated epithelial cells in the trachea and phagocytosis by alveolar macrophages impedes the pulmonary delivery of drugs [79].

Among the various strategies for improving pulmonary drug delivery, those that involve agglomerates of NPs with low mass density (<0.4 g/cm^3) and high geometric diameter (>5 μm) have been attracting increasing interest [80]. The low mass density of NP agglomerates with a small aerodynamic diameter prevents them from undergoing mucociliary clearance and facilitates their deposition in deep regions of the lung, whereas their relatively large geometric size causes them to escape phagocytosis by alveolar macrophages [79]. Upon deposition to the lower respiratory tracts of the lungs and exposure to a humid environment, the nanocomposite agglomerates dissolve and rapidly release the NPs [81]. Table 3 presents examples of agglomerates of NPs that are responsive to environmental stimuli for pulmonary insulin delivery [81–83].

Karathanasis et al. produced agglomerates of insulin-containing liposomes that were linked by concanavalin A (Con A), which is a glucose-binding protein [81]. They administered the Con A-linked agglomerates into the lungs of hyperglycemic rats *via* the pulmonary route. Upon exposure to the systemic glucose that was transported into the lung *via* the sodium-glucose transporter [84], the Con A interliposome links of agglomerates were readily cleaved, with possible disruption of liposomal membranes, releasing their encapsulated insulin, reducing systemic glucose levels.

Karathanasis et al. also developed agglomerates of liposomes that were crosslinked by dithiobis(succinimidyl propionate) (DTSSP), which is a disulfide-containing crosslinker that can be cleaved by cysteine by thiol–disulfide exchange [83]. The intratracheal instillation of the DTSSP-crosslinked agglomerates into diabetic rats rapidly reduced glucose levels, while subsequent administration of cysteine triggered an additional reduction of glucose level, suggesting an increase in the release of insulin from the carriers.

According to the relevant literature, the success of pulmonary delivery is largely controlled by the site of drug deposition, as different regions of the lung have different barrier properties [74]. Additionally, the dose of the drug that is inhaled *via* the pulmonary route may vary with the effectiveness of lung functions of the patients [85]. Hence, the advancement of inhalation technology to improve

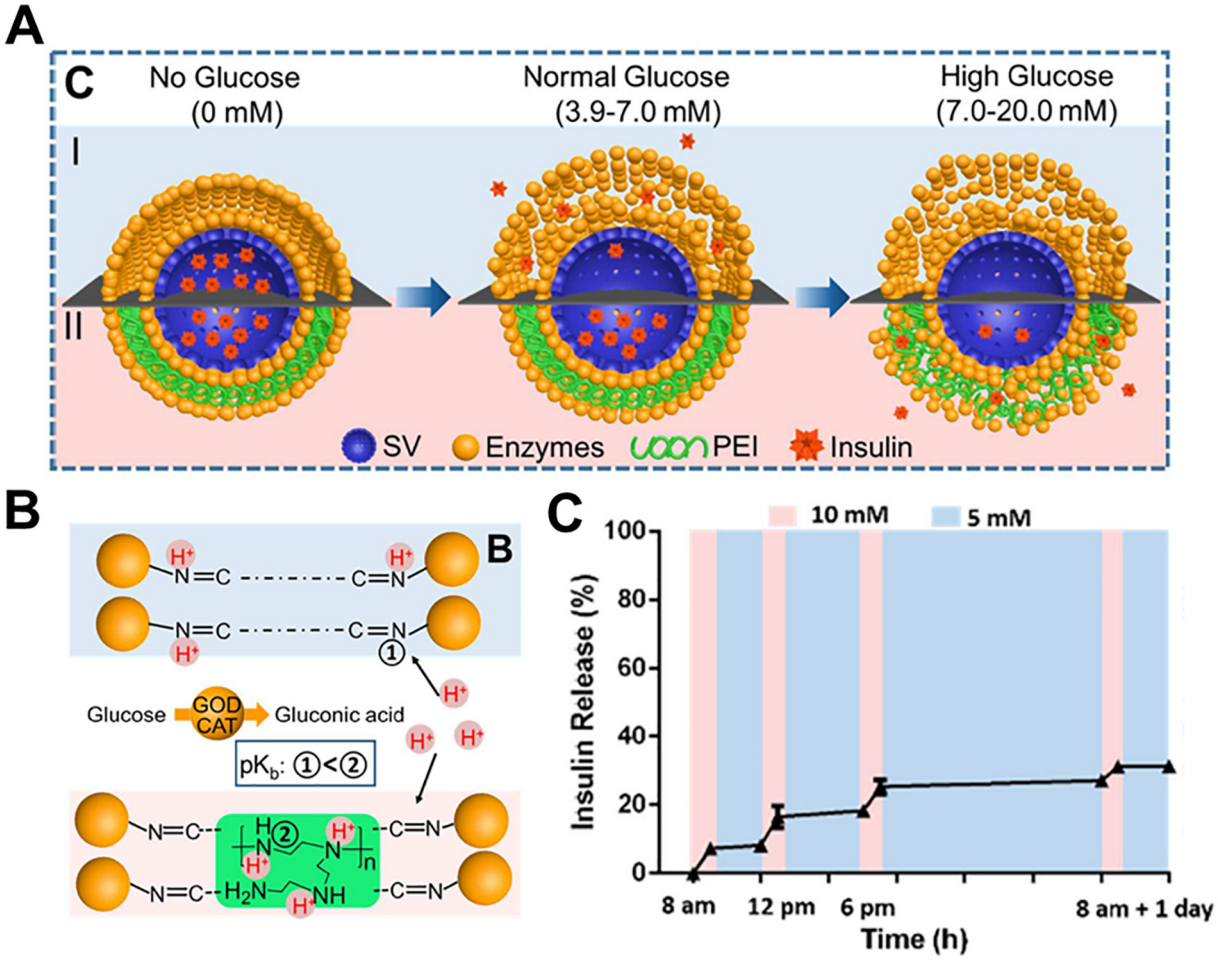

Fig. 5. Diabetic glucose level-responsive carrier *via* subcutaneous administration. (A) Differences in insulin release mechanisms between traditional glucose-responsive insulin release systems (I) and physiological glucose-responsive systems (II). (B) Mechanisms of glucose-responsive insulin release in enzyme system (top, blue area) and enzyme-PEI system (bottom, pink area). (C) Release profiles of insulin from enzyme-PEI system in solutions of various concentrations of glucose. SV: silica vesicles. Reprinted with permission from [89].

control over the medication that reaches the intended site of deposition is desirable.

5. Subcutaneous delivery of insulin

The quest to eliminate the need for daily multiple injections for subcutaneous insulin delivery and to replace them with an injectable, stimuli-responsive insulin delivery system that would significantly lower injection frequency has gained much attention. Apart from physiological factors such as glucose level, pH, or temperature as triggers of insulin release from stimuli-responsive systems, many external signals, including ultrasound, a magnetic field, and near-infrared (NIR) light, have been investigated as alternatives for initiating a sustained release of therapeutics for long-term diabetes treatment [86–98] (Table 4).

5.1. Glucose-responsive systems

Glucose-responsive systems, which are designed for the closed-loop delivery of insulin, can sense an increase in blood glucose levels and respond to them to control the release of insulin load. These closed-loop insulin delivery systems mimic the physiological response to changes in blood glucose levels, minimizing the potential for hypoglycemia. Many stimuli-sensitive materials that incorporate a glucose-sensing moiety of an enzyme (glucose oxidase; GOx) [86–89] or small molecule (PBA) [90–92] have been developed to respond to glucose.

5.1.1. Enzyme-dependent carriers

GOx, which is the most frequently used glucose-sensing moiety, can enzymatically convert glucose into gluconic acid in biological environments. The oxidation of glucose by GOx is typically accompanied by local consumption of O_2 and rapid generation of H_2O_2, deactivating GOx [59]. To enhance the activity of GOx, catalase (CAT) has been incorporated in glucose-responsive systems because it can convert H_2O_2 to water and O_2 (Fig. 4A) [87].

Gu et al. developed an injectable CS MP system that was crosslinked by tripolyphosphate to encapsulate GOx- and CAT-loaded nanocapsules and insulin, as a glucose-responsive insulin delivery system [87]. Under hyperglycemic conditions, the as-developed CS MPs swelled greatly on account of the enzymatic conversion of glucose into gluconic acid, which protonated the CS polymers, generating electrostatic repulsive forces within the carrier matrices (Fig. 4B). Accordingly, the CS MP system that included the enzyme nanocapsules could release insulin at basal release rates under normoglycemic conditions and at higher rates under hyperglycemic conditions. When administered subcutaneously in diabetic mice, these CS MPs facilitate insulin release in response to changes in blood glucose levels, providing a tighter glycemic control (Fig. 4C and D).

Owing to the high activity of the glucose-sensing enzymes (GOx and CAT), which causes very large pH changes and swelling of the matrices of the carriers, most reported glucose-responsive systems release a significant amount of their loaded insulin even at glucose levels that are below the normoglycemic levels (<7 mM), potentially causing hypoglycemia [89]. An interesting glucose-responsive insulin delivery system that can mimic the physiological insulin secretion behavior to control the release of insulin has been proposed (Fig. 5A) [89]. This insulin delivery system was prepared by layer-by-layer coating of an enzyme (GOx and CAT) — polymer (polyethylenimine; PEI) on mesoporous silica NPs that contained insulin. The enzyme—polymer multilayers forming a shell on the surface of the silica NPs were crosslinked by glutaraldehyde and acted as a valve to control the release of their loaded insulin in response to blood glucose levels. PEI polymer layers, which have a stronger proton affinity than enzyme layers, buffered the huge pH

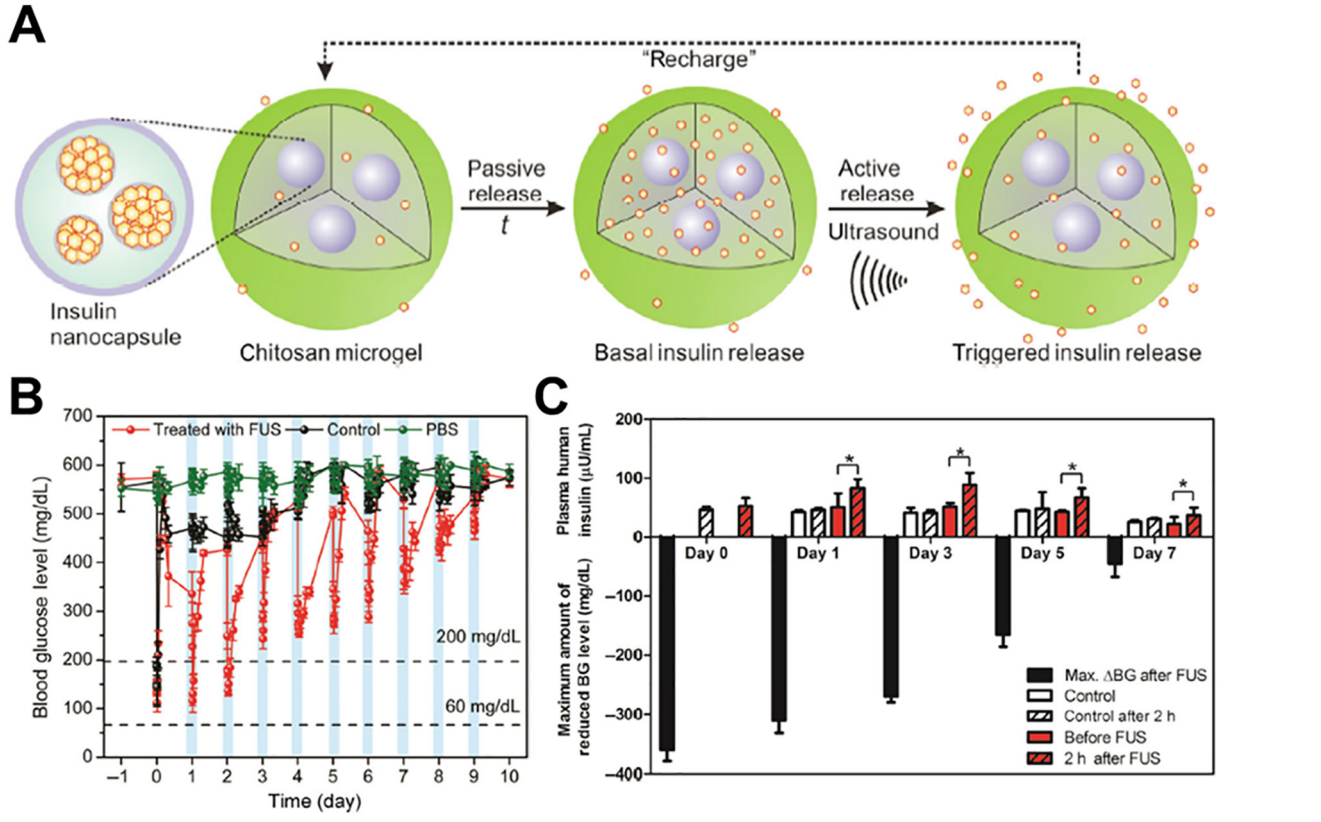

Fig. 6. Ultrasound-facilitated insulin delivery *via* subcutaneous administration. (A) Working mechanisms of CS MPs that incorporate with insulin-loaded PLGA NPs as an ultrasound-facilitated delivery system. (B) Blood glucose levels of diabetic mice after one dose of PBS or CS MPs is injected subcutaneously with or without focused ultrasound (FUS) treatment. (C) Changes in plasma insulin concentration and absolute reductions in blood glucose levels over time after initial and after each subsequent FUS application. BG: blood glucose. Reprinted with permission from [95].

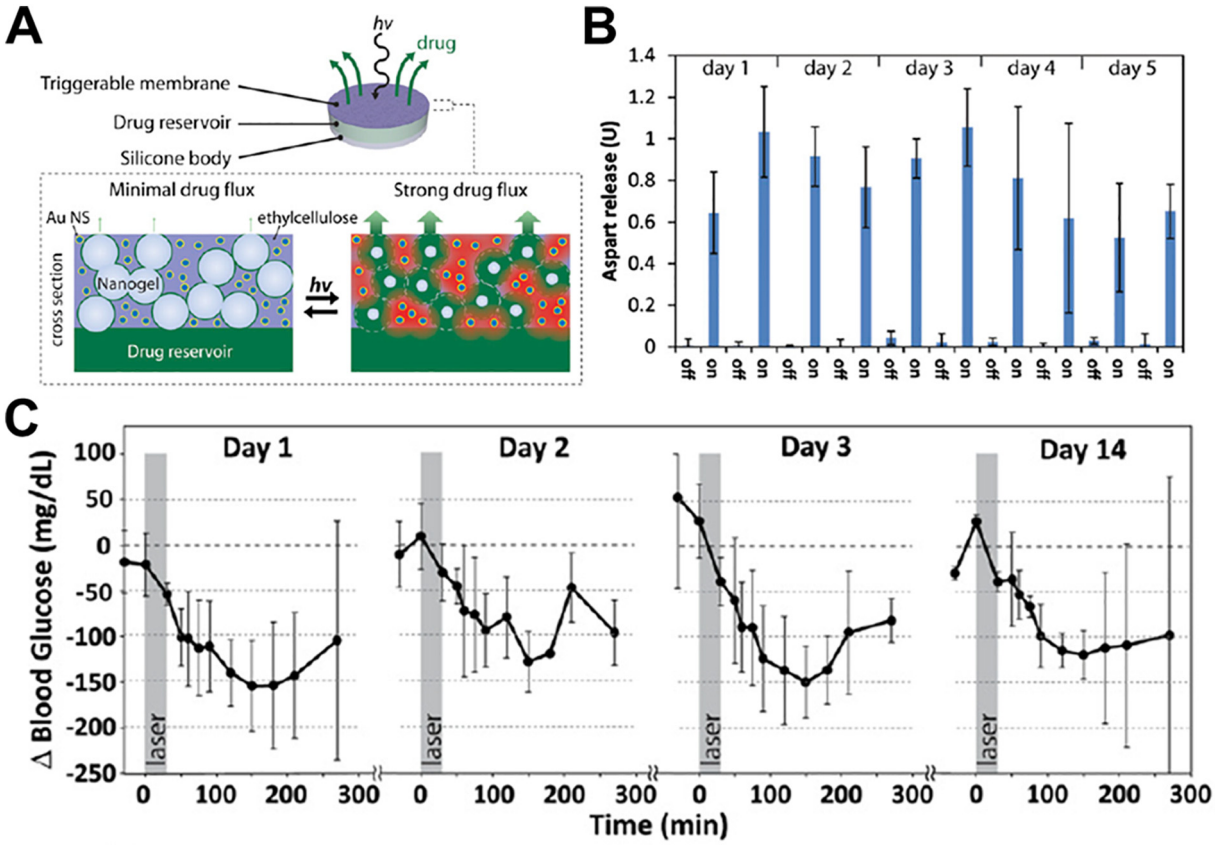

Fig. 7. NIR-activated insulin delivery *via* subcutaneous administration. (A) Proposed NIR-activated insulin delivery device (top) and cross-section of its triggerable membrane (bottom). (B) Insulin release from NIR-activated device over 30-min dosing cycles (570 mW/cm^2). (C) Blood glucose levels after repeated dosing at fixed irradiance (gray box; 30 min duration; 570 mW/cm^2) on four occasions over 14 days. Au NS: gold nanoshells. Reprinted with permission from [98].

changes by the enzyme-catalyzed conversion of glucose into gluconic acid, reducing the swelling of the enzyme multilayer shells, thereby elevating the insulin-release threshold (Fig. 5B). The insulin release was switched "ON" when the blood glucose concentration exceeded the normoglycemic level (>7 mM) and switched"OFF" at a normal glucose level (5 mM). The "ON—OFF" switching was repeatable (Fig. 5C).

5.1.2. PBA-based carriers

Another frequently used method for triggering the release of insulin from the glucose-responsive carriers is based on a non-enzymatic glucose-sensing moiety, PBA. PBA can form borate/diol complexes with a polyol, such as poly(vinyl alcohol) (PVA), which can be readily disrupted in the presence of glucose, which is also a polyol [59]. Table 4 lists examples of the use of PBA-containing systems for the subcutaneous delivery of insulin. As insulin is released from these glucose-responsive carriers by similar mechanisms, only one example is discussed here.

Wu and coworkers prepared a porous PLGA MP system that was loaded with insulin and then shell-coated with multilayers of PVA and a PBA-containing copolymer [92]. Upon exposure to glucose, the borate/diol complexes in the multilayers were disrupted, owing to the competitive binding of free glucose to the PBA-containing copolymer, causing the release of insulin from the PLGA MPs. A single subcutaneous injection into diabetic mice revealed that the multilayer PLGA MPs could effectively maintain blood glucose levels within the normal range for at least 18 days, revealing their potential as a smart insulin delivery system for the treatment of diabetes.

Table 5
Comparison of alternative insulin administration strategies.

	Oral administration	Intranasal administration	Pulmonary administration	Subcutaneous administration
Advantages	Patient-friendly Non-invasive; painless Convenient for repeated administration	Non-invasive; painless Large absorption area Low hepatic first-pass metabolism	Non-invasive; painless Wide absorption area Low hepatic first-pass metabolism	Less invasive High bioavailability Low hepatic first-pass metabolism
	Direct action in liver, having similar dynamic to endogenous insulin release	Rapid blood flow High vascularity of epithelial layer	Less enzymatic degradation Rapid blood flow Thin and high vascularized epithelium of alveoli	
Limitations	Hepatic first-pass metabolism Poor bioavailability because of harsh GI environment (mucus barrier, low pH, enzymatic degradation)	Short residence time Rapid mucociliary clearance Limited nasal volume	Unpredictable drug deposition Uncontrolled breath patterns Fast removal rate (mucociliary clearance and phagocytosis)	Pain; discomfort Scaling-up of smart delivery systems

5.2. pH/temperature-responsive insulin delivery

The use of an injectable pH/temperature-responsive hydrogel system for controlled subcutaneous insulin delivery has been proposed [93]. That hydrogel system consisted a pentablock copolymer of PAE-PCL-PEG-PCL-PAE, in which poly(β-amino ester) (PAE) was used as a pH-sensitive moiety to conjugate to the temperature-sensitive biodegradable triblock copolymer of poly(ethylene glycol)-poly(ε-caprolactone) (PCL-PEG-PCL). The cationic nature of the PAE segments of the copolymer was exploited to load anionic insulin by electrostatic complexation at low pH and to release the insulin in a sustained manner by decomplexation through the degradation of the copolymer at physiological pH and temperature. Following subcutaneous injection, the pentablock copolymer exhibited a sol-gel transition, and the release of insulin from the complex gel was sustained, lasting for about 15 days.

5.3. Ultrasound-facilitated insulin delivery

An ultrasound-facilitated drug delivery strategy, using a CS MP system incorporating insulin-loaded PLGA NPs that can release therapeutics in a sustained manner, has been experimentally demonstrated for treatment of diabetes [95]. Upon subcutaneous administration in a STZ-induced diabetic mouse model, the encapsulated insulin passively diffuses from the PLGA NPs, being temporarily stored in the matrices of CS MPs; following each ultrasound treatment, the stored insulin is rapidly released (Fig. 6A). The pulsatile release of insulin from the CS MPs is realized by the repeated application of ultrasound (Fig. 6B), resulting in the long-term release of therapeutics, providing glycemic control for up to one week (Fig. 6C).

5.4. Magnetic field-actuated insulin delivery

A magnetic field-actuated insulin delivery system of CS/alginate MPs that contained iron oxide NPs and insulin was formulated for subcutaneous implantation [97]. Upon exposure to an oscillating magnetic field, the magnetite NPs that were encapsulated within the as-formulated CS/alginate MPs began to undergo an oscillatory motion, which significantly enlarged the porosity of their polymeric matrices, remotely initiating the release of insulin. *In vivo* experiments revealed that the insulin that was released from the test MPs retained its activity.

5.5. NIR-activated insulin delivery

Another interesting strategy for the controlled subcutaneous delivery of insulin is based on an NIR-activated device that comprises a drug reservoir that is covered by an impermeable ethylcellulose membrane that contains gold NPs [98]. Upon the application of NIR light, the contained gold NPs are immediately heated, forming a porous structure on the impermeable membrane, allowing a rapid release of insulin aspart, which is a fast-acting insulin analog (Fig. 7A and B). Following subcutaneous implantation in diabetic rats, the NIR-activated device can be modulated by the intensity and timing of repeated irradiation, achieving glycemic control with reproducible dosing for two weeks (Fig. 7C).

Relative to the conventional subcutaneous treatment with insulin, the smart drug-delivery systems that release therapeutic drugs at the right time in response to physiological or exogenous stimuli have greater therapeutic efficacy and higher compliance from diabetic patients [99].

6. Future perspectives

Micro- and nanoencapsulation-based insulin delivery carriers or devices with stimuli-triggering mechanisms have been established to have remarkable therapeutic effectiveness for potential clinical applications. Despite the fact that insulin delivery by alternative routes has been demonstrated to be superior to conventional subcutaneous injections, the development of an effective and safe delivery system for each insulin administration route remains challenging (Table 5).

To avoid inducing hypoglycemia, carrier systems must not allow the burst release of insulin. The effective management of blood glucose level depends on precise control of the dose of insulin that is released from stimuli-responsive systems. A glucose-responsive-based closed-loop system may act as a real-time glucose sensor and a non-invasive actuator in the control of trigger signals to activate insulin release. Another challenge is to protect the pharmaceutical activity of the delivered insulin in the stimuli-responsive formulations when they are administered into the complex physiological environment. Immobilizing enzyme inhibitors in carrier systems may solve this problem, increasing the bioavailability of the delivered therapeutics.

The scaling-up of such micro- and nanodelivery systems and their toxicity and biocompatibility should be carefully evaluated. Formulations that satisfy the clinical need for fewer diabetic complications and better patient compliance must also be considered. Future investigations should focus on the screening of effective and safe formulations and excipients used, with a view to achieving their translation from the bench to the bedside. Finally, the long-term efficacy of these stimuli-responsive systems must be demonstrated in large animals and in humans before they can be used for insulin delivery.

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