



Review article

Recent progress in design and preparation of glucose-responsive insulin delivery systems



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ABSTRACT

Diabetes, as a global threat nowadays, causes the wide usage of exogenous insulin. Subcutaneous injection is the traditional insulin administration in the diabetes treatments and is inconvenient and painful, which results in potential side effects of hypoglycemia. Scientists have made great efforts to develop alternatives. As the promising solutions, smart insulin delivery systems fabricated with glucose-responsive compounds attract considerable attention in the field of diabetes treatments. This review discusses the classifications, properties, response mechanisms of the glucose-responsive compounds, glucose oxidase and concanavalin A and recent progress of glucose-responsive insulin delivery systems.

1. Introduction

Diabetes has shown increasing risks of death and disability in recent years. According to the statistics from IDF (international diabetes federation) diabetes atlas, in 2017, over 425 million people suffered from diabetes worldwide and it was predicted that diabetes would affect 629 million people's life in 2045 [1]. Thus, how to treat diabetes becomes the global hotspot and receives considerable attention from both academia and industry fields.

Drug intervention is fairly common in diabetes treatments and exogenous insulin is one of the most important antidiabetic medicines [2]. So far, the insulin administration widely adopted nowadays has been based on frequent subcutaneous injection which has resulted in pain and possible hypoglycemia [3]. The pump system for continuous subcutaneous infusion with the controllable insulin flow acts as the second choice for diabetic patients. However, the high price, potential risks of skin infection and lack of portability impede the wider application [4–7]. Recently, emerging technologies are introduced in the insulin administrations in order to avoid pain [8]. Typical insulin

administrations for reducing pain include the oral administration, nasal administration, pulmonary administration, transdermal administration as well as low-frequency injection administration [9–13].

To avoid hypoglycemia and improve controllability of insulin delivery, glucose-responsive compounds for fabrication of smart insulin delivery systems stimulate interest of the scientific world [14]. A smart insulin delivery system is always comprised of a glucose-responsive unit and an insulin carrier [14]. A glucose-responsive unit acts as the sensor to detect the blood glucose levels (BGLs) [14]. Subsequently, the performance changes of an insulin carrier, such as hydrophilicity, the crosslinking degree and pH, are induced, which regulate the releasing rate of insulin in the carrier [15,16].

The combination of pain-reducing technologies and glucose-responsive compounds shows an attractive future in designing smart insulin delivery systems with less pain. Fig. 1 illustrates the general working mode of smart insulin delivery systems and the pain-reducing technologies [14]. Therefore, this review discusses advantages and disadvantages of the pain-reducing insulin administrations in detail and systematically summarizes the classifications, properties and response

Abbreviation list: (Abbreviation, Full name); BGL, Blood glucose level; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; MNP, Microneedle patch; CPP, Cell penetrating peptide; PBA, Phenylboronic acid; GOx, Glucose oxidase; Con A, Concanavalin A; DDOPBA, 4-(1,6-Dioxo-2,5-diaza-7-oxamyl) phenylboronic acid; NIPAAm, N-isopropylacrylamide; AAPBA, 3-Acrylamidophenylboronic acid; AmECFPBA, 4-(2-Acrylamidoethylcarbamoyl)-3-fluorophenylboronic acid; 3-APBA, 3-Aminophenylboronic acid; HMDA, 1,6-Hexamethylenediamine; FAD, Flavin adenine dinucleotide; MOF, Metal-organic framework; CAT, Catalase; HRP, Peroxidase; NPBA, Nitrophenylboronic acid; PVA, Poly(vinyl alcohol); RBC, Red blood cell; ZIF, Zeolitic imidazolate framework; INC, Insulin-loaded nanocarrier; HA, Hyaluronic acid; PV, Polymeric vesicle; NI, 2-Nitroimidazole; GRV, Glucose-responsive vesicle; MBG, Mesoporous bioactive glass; QD, Quantum dot; NIPMAAm, N-isopropylmethacrylamide

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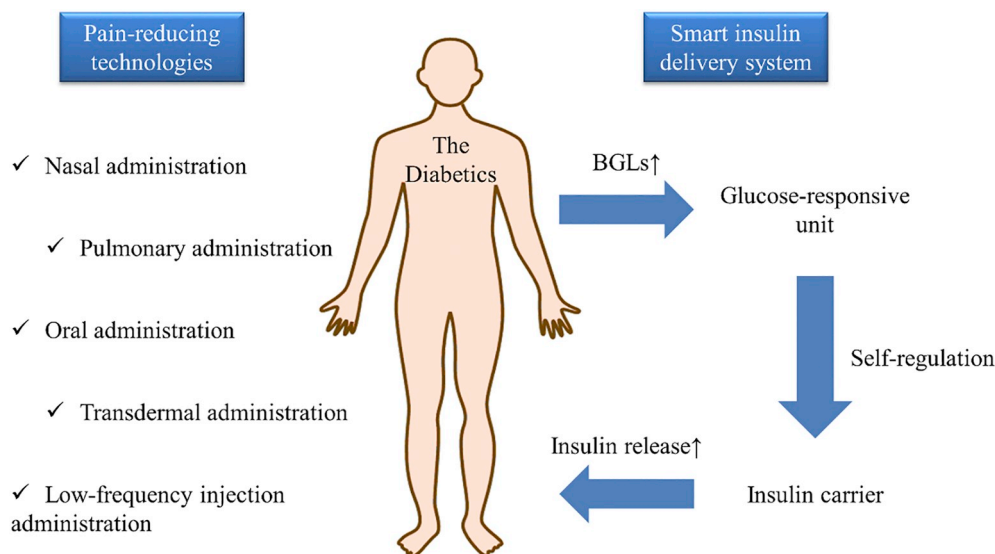


Fig. 1. The general working mode of smart insulin delivery systems and the pain-reducing technologies [14].

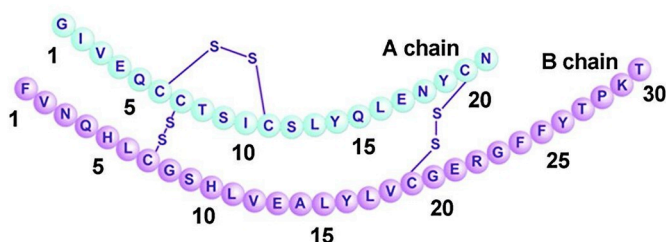


Fig. 2. The amino acid sequence of human insulin. (Adapted with permission from ref. [28]. Copyright 2014 The Royal Society of Chemistry.)

mechanisms of glucose-responsive compounds. Furthermore, recent progress of glucose-responsive insulin delivery systems is highlighted with a comprehensive perspective for diabetes treatments in the future.

2. Diabetes, insulin and insulin delivery systems

It is necessary to understand the relationship between diabetes and insulin before an insulin delivery system is designed. Two leading challenges for insulin delivery are discussed.

2.1. The relationship between diabetes and insulin

Diabetes mellitus, or simply called diabetes, is a metabolic disorder characterized by hyperglycemia [17]. Diabetes is caused by impaired insulin secretion or insulin resistance [17]. Insufficient supply of insulin directly leads to disturbance of BGLs, fat as well as protein metabolism [17]. In a long run, different complications gradually appear, such as retinopathy, nephropathy, neuropathy, diabetic foot, bone fragility, stroke, peripheral artery disease and congestive heart failure [18–21].

Most of the diabetes patients are diagnosed as type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) [17]. T1DM features absolute deficiency of insulin caused by the reduction of islet β -cells mass, while T2DM is usually associated with obesity due to overnutrition [22]. These two types of diabetes similarly result in hyperglycemia and, subsequently, raise bodies' demand on exogenous insulin in order to keep the metabolic balance of blood glucose [23–27]. Exogenous insulin has been long challenged by two problems.

The first challenge is that the strategy for insulin delivery is extremely limited. A human insulin molecule is constructed by 51 amino acids (Fig. 2) with a total molecular weight of 5808 Da and possesses

complicated three-dimensional structures [28]. High temperature, enzymatic degradation and poor permeability across biological barriers make subcutaneous injection the only commercial approach for the insulin administration nowadays [29–31]. However, diabetic patients commonly feel painful due to frequent injection [29]. Moreover, adverse consequences also exist, such as hypoglycemia, lipoatrophy and lipohypertrophy [32]. Therefore, it is necessary to develop alternative technologies for insulin delivery.

The second challenge is how to stabilize BGLs of diabetic patients in a long term with the proper dose of exogenous insulin, namely to avoid both hyperglycemia and hypoglycemia. Porte et al. [33] reported that the insulin secretion of a healthy body followed a “two-pool” mechanism. One pool could release enough insulin in a short period of time to quickly reduce postprandial blood glucose, while the second pool could produce and release insulin sustainably for homeostasis of the fasting blood glucose [33]. Obviously, it is inconvenient to mimic the natural “two-pool” system by simply injecting insulin, even though different types of insulins have been developed for this aim (Table 1) [34,35]. Furthermore, the dose of insulin for each injection is highly dependent on personal experience and the operation method [36]. So how to make the insulin delivery process convenient and controllable is the key point for this challenge.

2.2. Insulin delivery systems

As the solutions for the first challenge, routes for insulin delivery through the intestine, nasal cavity, lung and skin are respectively studied [37,38]. The advantages and drawbacks of different insulin administrations nowadays are summarized in Fig. 3 [37]. Consequently, the patterns of insulin delivery systems and methods of modification vary from route to route.

For subcutaneous injection, it is frequent pricking that leads to pain and side effects [37]. Therefore, to lower the frequency, insulin delivery systems are aimed to prolong the insulin releasing period. For example, insulin-loaded microspheres could be simply delivered into human bodies via injection, but the releasing period would be much longer in comparison with insulin of direct injection [39]. Other insulin-loaded injectable particles [40] and injectable hydrogels [13,41] also follow the similar working mechanism.

Fabrication of painless needles is a feasible approach to avoid painful experience in insulin injection as well [36]. As the typical technology, microneedle patches (MNPs) for transdermal insulin delivery possess much thinner and shorter needles to minimize pain [42].

Table 1
Classification of insulins [34,35].

Insulin type	Typical brands of insulin	Target
Rapid-acting insulin	Insulin lispro and insulin aspart	Avoid postprandial hyperglycemia
Short-acting insulin	Humulin R and Novolin R	Avoid postprandial hyperglycemia
Intermediate-acting insulin	Isophane insulin and insulin zinc	Control fasting BGLs
Long-acting insulin	Insulin glargine, insulin zinc-crystalline and insulin detemir	Control fasting BGLs

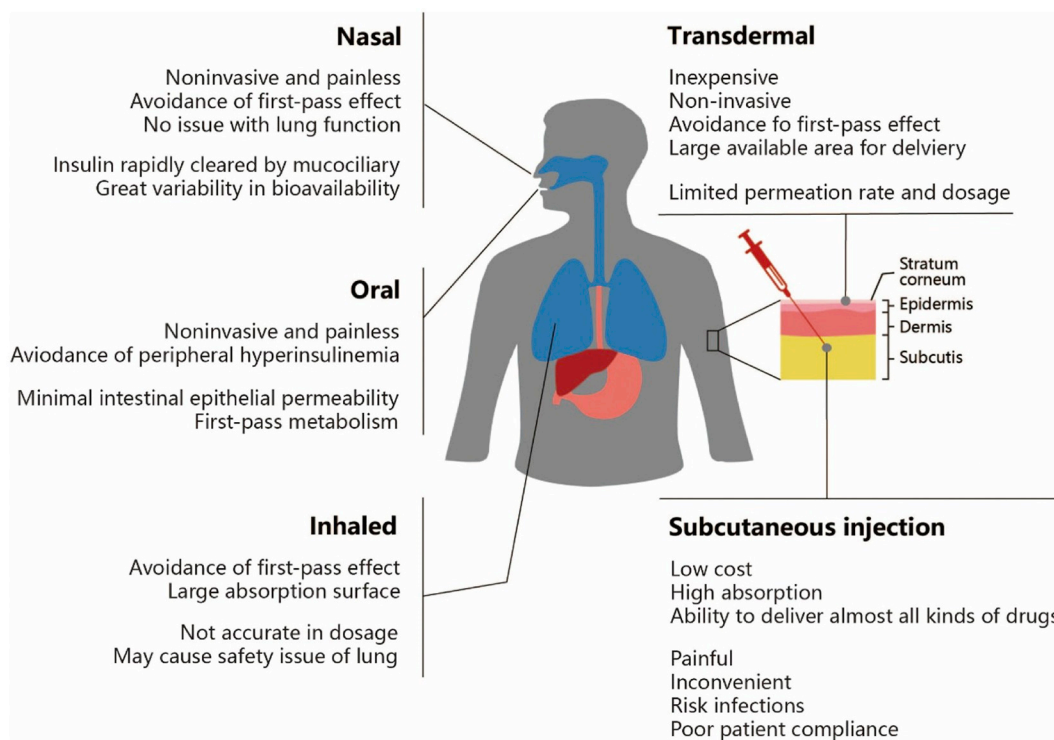


Fig. 3. The advantages and drawbacks of different insulin administrations. (Adapted with permission from ref [37]. Copyright 2018 Elsevier.)

Table 2
Classification of glucose-responsive compounds [14,59,60].

Property	Phenylboronic acid (PBA)	Glucose oxidase (GOx)	Concanavalin A (Con A)
Source	Synthetic chemicals	Natural proteins	Natural proteins
Molecular weight	10^2 – 10^3 Da	130–186 kDa	25.5 kDa
Glucose-responsive mechanism	Specific reaction with glucose	Enzymatic reaction	Glucose binding behavior
Strong interferents	D-fructose, D-galactose and other 1,2-cis-/1,3-diols	/	D-mannose and D-fructose

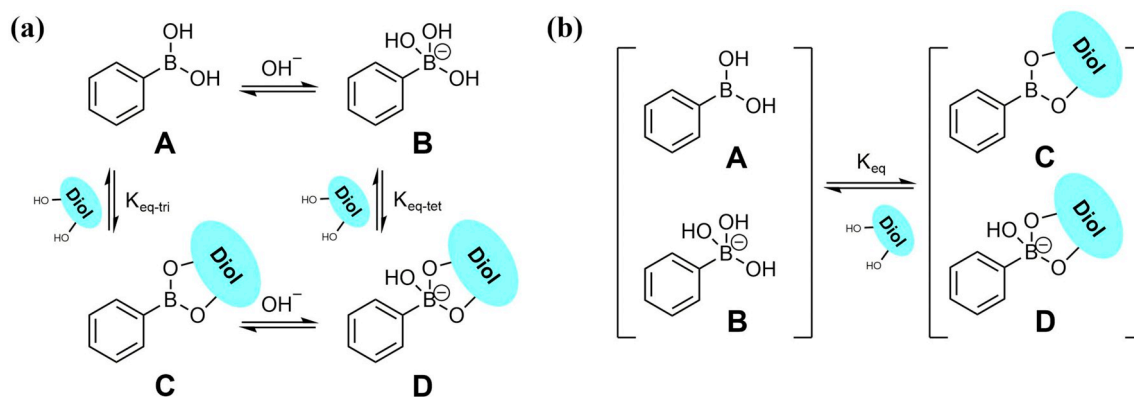


Fig. 4. (a) The equilibrium between charged and uncharged phenylboronic acid and their esters [63]. (b) The equilibrium between phenylboronic acids and their esters. K_{eq-tri} , K_{eq-tet} and K_{eq} are all equilibrium constants and K_{eq} is named as overall association constant [63].

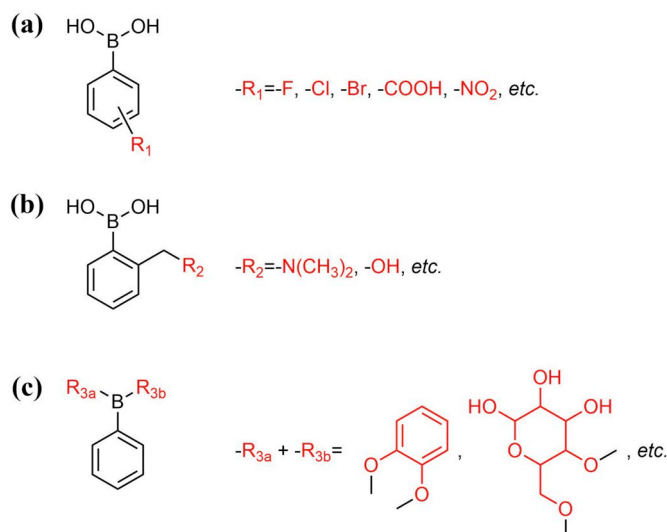


Fig. 5. Examples of different PBA and its derivatives. (a) PBA with electron-withdrawing groups, (b) PBA with “B-N” coordination bonds or “B-O” coordination bonds and (c) PBA/diol complexes.

MNPs could be prepared by metals, silica and polymers, in which polymeric MNPs are accessible for chemical modification and carry chemical blood-glucose sensors for smart insulin delivery [12]. The major obstacle for polymeric MNPs is the low capacity of insulin [43].

Oral administration is another common approach for drug delivery [44]. However, how to avoid enzymatic degradation and achieve better intestinal permeability are the main challenges for oral insulin [45]. Insulin-loaded carriers like particles and hydrogels are usually modified with protective layers and absorption enhancers [8,46]. For example, Shan et al. [47] reported a novel nanoparticle platform for efficient oral delivery of insulin. Cell penetrating peptide (CPP) was co-loaded with insulin in order to achieve efficient epithelial absorption and mucus permeation [47]. Fan et al. [48] designed an insulin-loaded chitosan nanoparticles modified by deoxycholic acid. Insulin could overcome multiple barriers of the intestinal epithelium by exploiting the bile acid pathway [48].

Apart from oral administration, other painless administrations such as nasal administration and pulmonary administration are also designed [8]. Insulin powders, which can be simply fabricated by packing insulin in supplementary materials, are commonly applied in both of these two administrations [49,50]. The key challenges for nasal insulin delivery are related to mucociliary clearance and poor insulin

permeability through the epithelium, so the insulin delivery systems could be modified by absorption enhancers and mucoadhesive polymers [51,52].

3. Glucose-responsive compounds, glucose oxidase and concanavalin A

When it comes to the second challenge—how to form a smart insulin delivery system of controllable insulin releasing behaviors, the key is to design stable units with the sensitive glucose response. These glucose-responsive units are based on glucose-responsive compounds, glucose oxidase and concanavalin A [53–58]. Phenylboronic acid and its derivatives are typical glucose-responsive compounds and could specifically react with glucose [14]. Glucose oxidase is the enzyme and could catalyze the glucose-related reaction [14]. Concanavalin A is the natural glucose-binding lectin and could be isolated from specific plants [14]. Table 2 lists the different properties and stimuli-responsive mechanisms of the glucose-responsive compounds, glucose oxidase and concanavalin A [14,59–62].

3.1. Phenylboronic acid and its derivatives

Phenylboronic acid (PBA) and its derivatives are the most widely used artificial compounds for fabrication of glucose-responsive units. PBA could shift between the uncharged trigonal form (hydrophobic) and the charged tetrahedral form (hydrophilic) under equilibrium [59]. Both forms of PBA could react with glucose or other 1,2-*cis*-/1,3-diols, but the charged PBA has better affinity with glucose [59]. Fig. 4 illustrates the equilibrium between these four molecules (A, B, C and D) above while Eqs. (1)–(3) give the definitions of K_{eq-tri} , K_{eq-tet} and K_{eq} [63].

$$K_{eq-tri} = \frac{[C]}{[A][diol]} \quad (1)$$

$$K_{eq-tet} = \frac{[D]}{[B][diol]} \quad (2)$$

$$K_{eq} = \frac{[C] + [D]}{([A] + [B])[diol]} \quad (3)$$

The biggest challenge to fabricate a glucose-responsive unit by PBA and its derivatives is how to improve their glucose-responsive performances at physiological pH ($pH = 7.4$) [62]. Three classes of methods are developed to conquer this problem (Fig. 5).

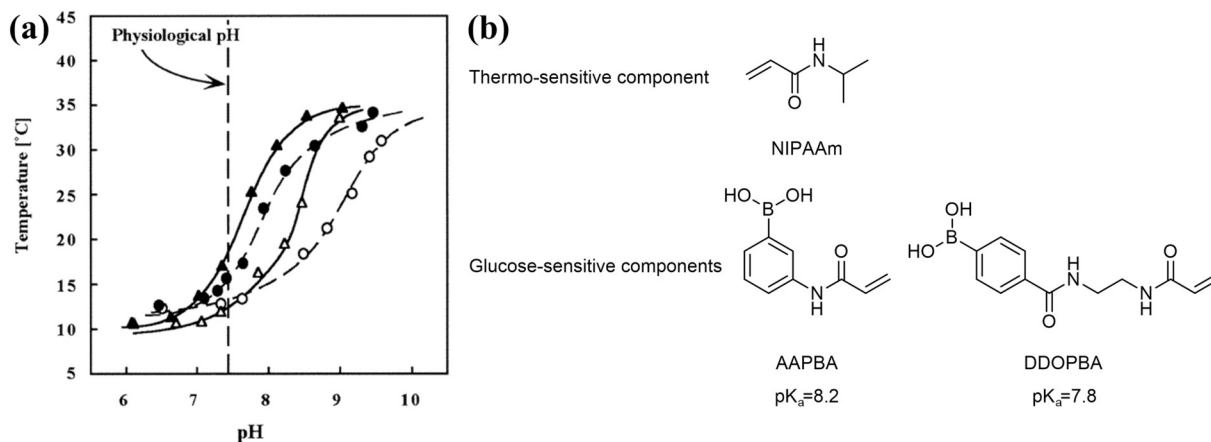


Fig. 6. (a) The relationship between LCST and pH of poly(NIPAAm_{89.5}-co-DDOPBA_{10.5}) (○, no glucose; ●, glucose concentration of 5 g/L) and of poly(NIPAAm_{90.5}-co-AA/PBA_{10.5}) (△, no glucose; ▲, glucose concentration of 5 g/L). (Adapted with permission from ref. [64]. Copyright 2003 American Chemical Society.) (b) The monomer structures of the copolymers [64].

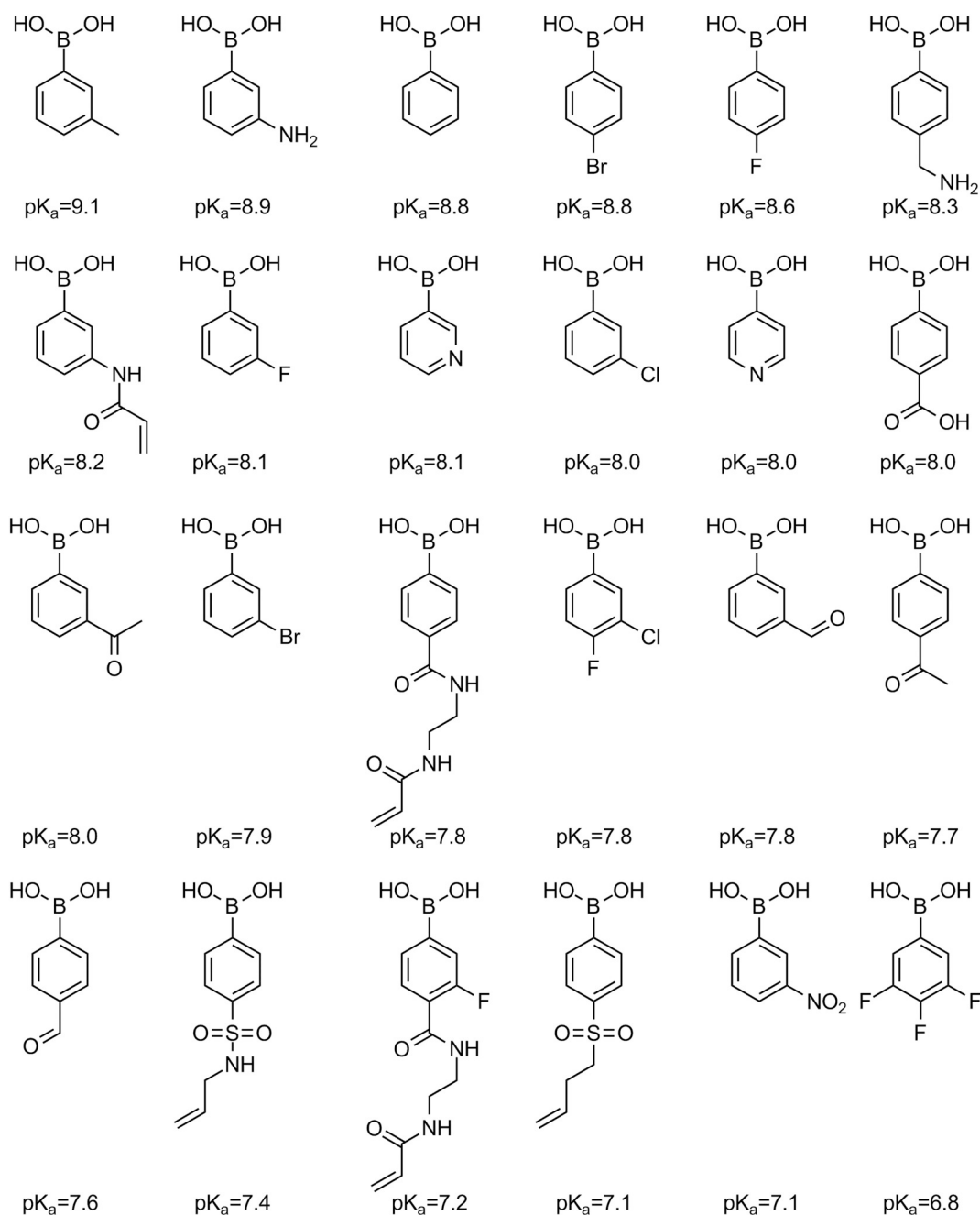


Fig. 7. The pK_a values of PBA and PBA derivatives [64,65,69–71].

3.1.1. Phenylboronic acid-based compounds with electron-withdrawing groups

Electron-withdrawing groups are introduced into the aromatic ring of PBA to make a PBA molecule more acidic, or in other words, to lower pK_a of the molecule (Fig. 5(a)). PBA with the lower pK_a value shows better affinity with glucose [59].

Matsumoto et al. [64] designed a novel molecule, 4-(1,6-dioxo-2,5-diaza-7-oxamyl) phenylboronic acid (DDOPBA), as a glucose-sensitive monomer, which was copolymerized with thermo-sensitive *N*-isopropylacrylamide (NIPAAm). The results showed that the glucose sensitivity was improved since the copolymer containing DDOPBA (pK_a = 7.8) moieties had a LCST (lower critical solution temperature) value closer to the physiological temperature compared with the 3-acrylamidophenyl-boronic acid (AAPBA, pK_a = 8.2) group under pH 7.4 (Fig. 6) [64].

Based on this strategy, 4-(2-acrylamidoethylcarbamoyl)-3-

fluorophenylboronic acid (AmECPBA), with a lower pK_a value of 7.2, was adopted to further adjust LCST [65]. 7.5 mol% AmECPBA endowed the polymeric hydrogel with excellent glucose-dependent swelling properties under the physiological temperature and pH [65].

Other substituent groups are also studied based on the Hammett equation [66], such as the sulfonyl group [67,68], nitro group [69], carboxyl group [64] and halogen atoms (–F, –Cl, –Br) [65]. Fig. 7 shows a series of pK_a values of PBA and PBA derivatives [64,65,69–71]. Fig. 8 gives a fitted straight line by relating pK_a values of PBA and substituted PBA compounds with substituent constants (σ) [64,65,69–71].

3.1.2. Phenylboronic acid-based compounds with “B–N” coordination bonds or “B–O” coordination bonds

Fig. 5(b) gives the typical structure of PBA derivatives with “B–N” coordination bonds or “B–O” coordination bonds. In Fig. 9, a

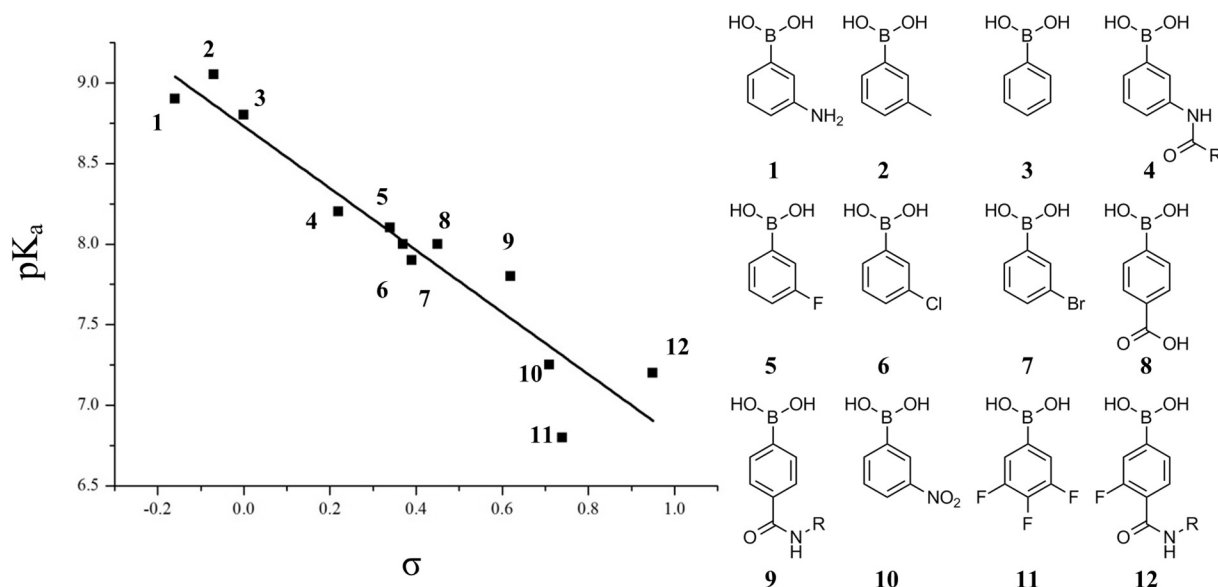


Fig. 8. The pK_a values of PBA and its derivatives as a function of substituent constants. Data of substituent constants are adopted from ref. [64, 65, 69] and pK_a values are adopted from ref. [65, 69–71].

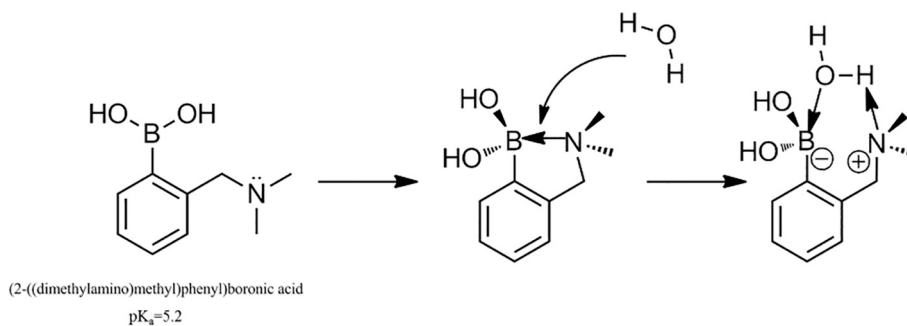


Fig. 9. The mechanism of solvent insertion in 2-((dimethylamino)methyl)phenylboronic acid [72].

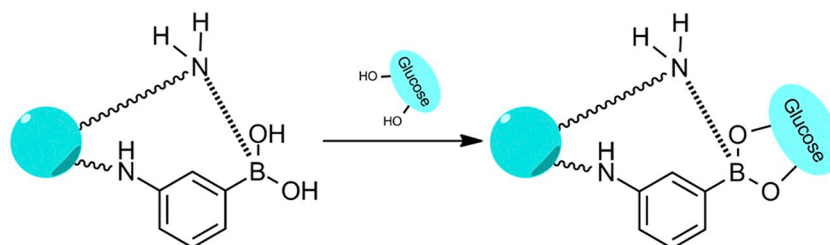


Fig. 10. Intermolecular “B–N” coordination between 3-APBA and HMDA [77].

dimethylaminomethyl group is introduced into the ortho-position of a PBA molecule to form the “Wulff-type” phenylboronic acid [72]. The intra-molecular coordination decreases the pK_a value of modified PBA to 5.2 due to the mechanism of solvent insertion [73,74].

Similarly, intermolecular “B–N” coordination could also reduce pK_a of PBA [75,76]. Ren et al. [77] designed a “B–N” complex based on 3-aminophenylboronic acid (3-APBA) and 1,6-hexamethylenediamine (HMDA). The complexes were covalently bonded on the macroporous epoxy resin and showed good affinity with *cis*-diol-containing compounds in a neutral medium (Fig. 10).

In comparison with the “Wulff-type” PBA, the modified PBA molecule with the “B–O” coordination bond possess a similar pK_a value and improved water solubility [78,79]. This type of PBA is called the improved “Wulff-type” PBA [78,79]. Chen et al. [80] described a hydrogel-based platform for 3D cell encapsulation. The gelation process

occurred successfully in the PBS of pH 7.4 (Fig. 11) by forming dynamic boronic ester bonds between the benzoxaborole and catechol [80].

3.1.3. Phenylboronic acid/diol complexes

Since PBA molecules could form boronic ester bonds with glucose and other diols, it is feasible to fabricate glucose-responsive hydrogels by grafting both PBA and diol moieties on the networks [59]. As shown in Fig. 5(c), derivatives of the catechol and saccharides were reported as the diol moieties [81,82]. Fig. 12 explains the general working mode of this type of systems [78]. Dissolution of the hydrogel networks occurs because the free glucose molecules compete with diol moieties in the hydrogel networks [78]. It is also remarkable that even these hydrogel systems containing the PBA moieties of high pK_a show glucose-responsive behaviors at physiological pH [81]. For example, Dong et al. [81] fabricated the hydrogel with AAPBA ($pK_a = 8.2$), but the glucose-

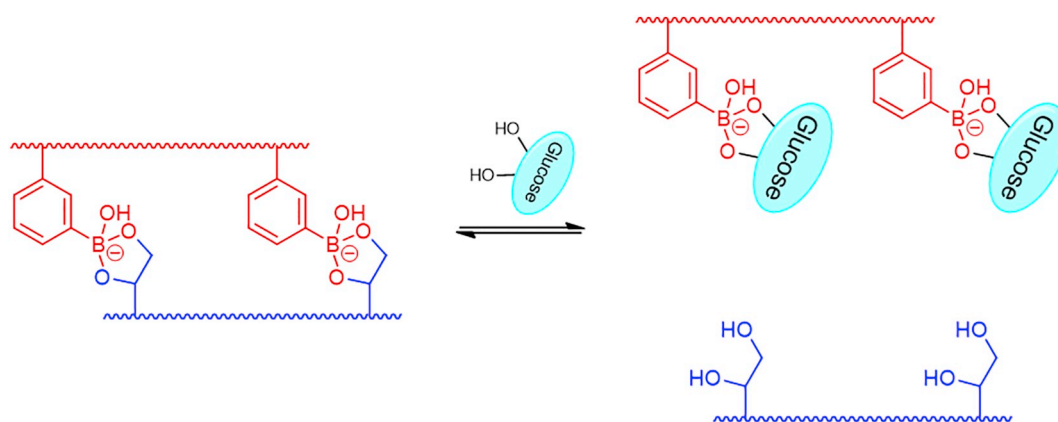


Fig. 11. Formation of dynamic benzoxaborole-catechol complexation in PBS (pH 7.4) [80].

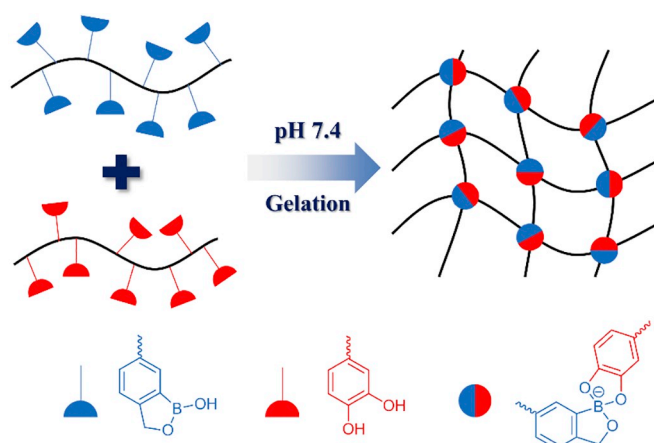


Fig. 12. The general working mode of the PBA/diol systems [78].

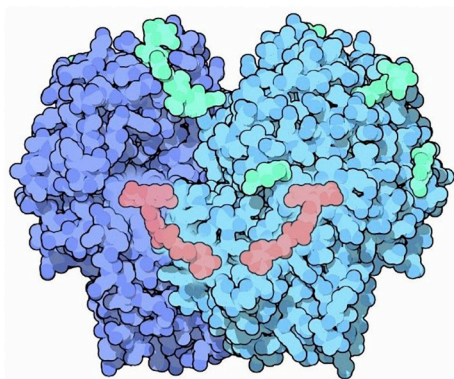


Fig. 13. The structure of GOx. Image from the RCSB PDB May 2006 Molecule of the Month feature by David Goodsell (DOI: https://doi.org/10.2210/rcsb_pdb/mom_2006_5) [85].

dependent drug releasing behavior existed in the hydrogel at physiological pH. The possible reason for this phenomenon was that a small part of the PBA moieties interacted with glucose. Then the interaction resulted in dissolution of the hydrogel.

3.2. Glucose oxidase

Glucose oxidase (GOx) is the most widely studied enzyme for monitoring BGLs. GOx exists widely in microorganisms, plants and animals [83]. GOx is comprised of two subunits bonded with two prosthetic groups of flavin adenine dinucleotides (FAD) respectively

[84]. Fig. 13 shows the structure of GOx [85], while Table 3 gives the basic properties of GOx [84].

GOx acts as a biocatalyst of high specificity in the oxidation reaction of D-glucose to D-glucono- δ -lactone [97]. D-glucono- δ -lactone subsequently converts into D-gluconic acid due to non-enzymatic hydrolysis [97]. Meanwhile, FAD takes two hydrogen atoms from glucose to form FADH₂, and FADH₂ reacts with O₂, the electron acceptor, to reach H₂O₂ [97]. Details of the mechanism of the GOx biocatalytic reaction are described in Fig. 14.

GOx shows excellent catalytic selectivity to β -D-glucose compared with the other saccharides and their derivatives [84]. The relative activities of GOx against different monosaccharides are listed in Table 4 [90]. In addition, D-glucose molecules existed as the open-chain structure, pyranoses and furanoses in equilibrium [90]. The predominant species, α -D-glucopyranose and β -D-glucopyranose, also indicated a remarkable difference in the GOx catalytic reaction [90]. The reaction rate based on α -D-glucopyranose is as fast as 0.64% of that based on β -D-glucopyranose [90].

Inactivity caused by high temperature, pH and enzymatic action is the biggest challenge when it comes to improving the performances of GOx as a glucose-sensitive unit. Wu et al. [98] adopted metal-organic frameworks (MOFs) as the protective layer to immobilize GOx, which successfully avoided activity loss in the trypsin or EDTA (1 wt%) solution. GOx could also be encapsulated in nanogels [99], hollow nanospheres [100] and vesicles [101] to avoid inactivity.

Inflammation of human tissues caused by H₂O₂ is another significant obstacle for GOx-based smart insulin delivery systems [99]. Most solutions are associated with catalase (CAT) [102], peroxidase (HRP) [98] and the other artificial peroxidases. For example, Qu et al. [103] encapsulated hemin in the GOx-based glucose-responsive hydrogels to obtain stable H₂O₂-consuming behaviors.

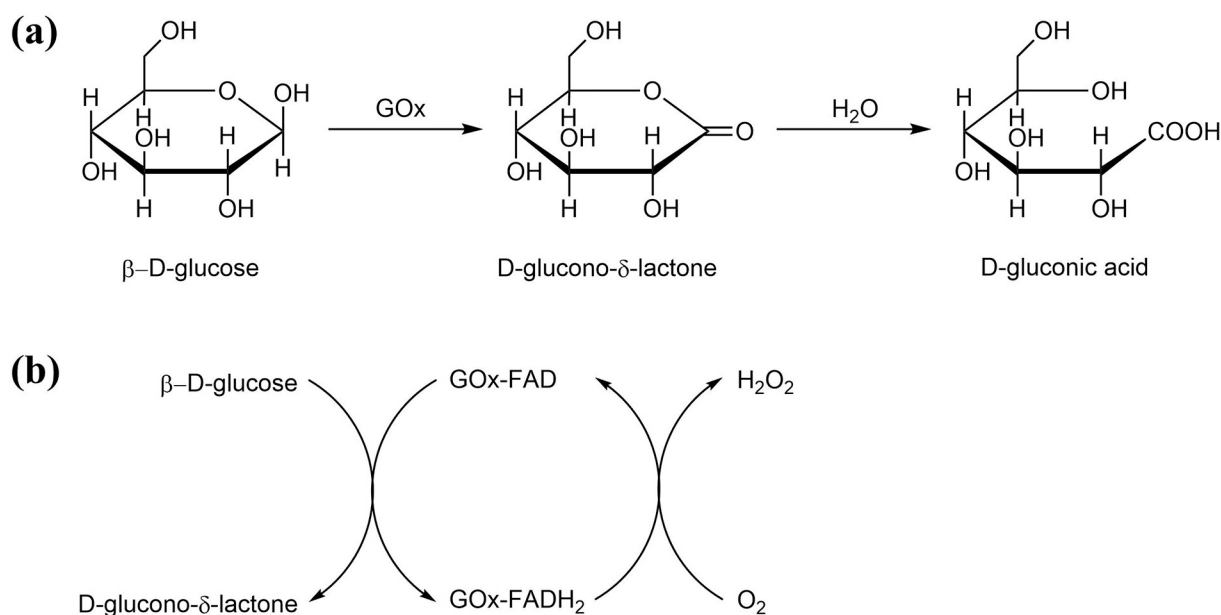
3.3. Concanavalin A

The third class of glucose-responsive compounds are related to glucose-binding proteins including gram-negative bacteria, apoenzymes and lectins [104]. Concanavalin A (Con A) is the most widely used lectin and each Con A molecule contains four sugar-binding sites at physiological pH [105–107].

Sugar-binding sites of Con A are capable of bonding with hydroxyl groups of α -D-mannopyranose, α -D-glucopyranose and the other non-reducing sugars at C-3, C-4 and C-6 [107]. Thus, the specifically bonding behaviors have been applied in detecting specific saccharides [108–112] and certain glycoprotein-containing cells [113–115]. For example, Li et al. [116] designed a novel glucose biosensor containing Con A and dextran. Con A and dextran dispersed and interacted on the surfaces of the probes, and then dissociated in the glucose-dextran competitive reaction when the glucose concentration raised [116].

Table 3The basic properties of GOx isolated from *Aspergillus niger*. (Adapted with permission from ref. [84]. Copyright 2002 Elsevier.)

Property	Description	Ref.
Molecular weight	130-186 kDa	[86–89]
Temperature range	20–50 °C	[84]
pH range	Around 5	[83,87,90]
Inhibitors	μM : Ag^+ , Hg^{2+} , Pb^{2+} and Cu^{2+} mM: hydrazine, hydroxylamine, phenylhydrazine, Arsenite, <i>p</i> -chloromercuribenzoate and phenylmercuric acetate	[83,91–93]
K_m (Michaelis constant, with respect to glucose)	10 mM: 8-hydroxyquinoline (11%), sodium nitrate (13%) and semicarbazide (20%) 198-248 mM (pH 5–7, 20–30 °C, oxygen) 110-120 mM (pH 5.6, 0–38 °C, oxygen) 50-74 mM (pH 5.5, 15–30 °C, oxygen) 33 mM (pH 5.6, 25 °C, oxygen)	[88,94–96]
Specific activity	80–172 (μmol glucose)/min/mg enzyme (pH 5.6, 25–37 °C)	[88,89]

**Fig. 14.** (a) The two-step reactions catalyzed by GOx. $\beta\text{-D-glucose}$ is converted to D-glucono- δ -lactone in the first step and D-glucono- δ -lactone is further hydrolyzed to D-gluconic acid in the second step [97]. (b) The mechanism of the first step [97].**Table 4**

The relative activities of GOx against different monosaccharides and their derivatives. (Adapted with permission from ref. [90]. Copyright 2005 Elsevier.)

Monosaccharide	Relative activity of GOx
$\beta\text{-D-glucose}$	100 (hypothesized)
$\alpha\text{-D-glucose}$	0.64
4- <i>o</i> -Methyl- D-glucose	15
6- <i>o</i> -Methyl- D-glucose	1
2-Deoxy- D-glucose	25–30
3-Deoxy- D-glucose	1
4-Deoxy- D-glucose	2
6-Deoxy- D-glucose	10
Mannose	0.2, 1
Altrose	0.16
Galactose	0.08
Xylose	0.03

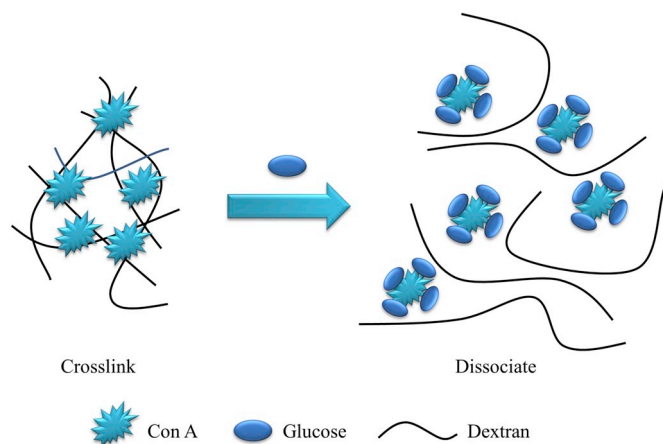
**Fig. 15.** The glucose-responsive mechanism of the glucose biosensor containing Con A and dextran [116].

Fig. 15 briefly shows the glucose-responsive mechanism of the glucose biosensor [116].

The most important problems for Con A are poor biocompatibility and instability [117]. Therefore, if Con A is applied as the glucose-responsive unit in a smart insulin delivery system, cytotoxicity and the decreasing glucose sensitivity should be seriously considered [117].

Immobilization of Con A was reported as a method to avoid these problems [58]. For example, Con A could be covalently bound in the hydrogel matrix against leakage into human bodies [118].

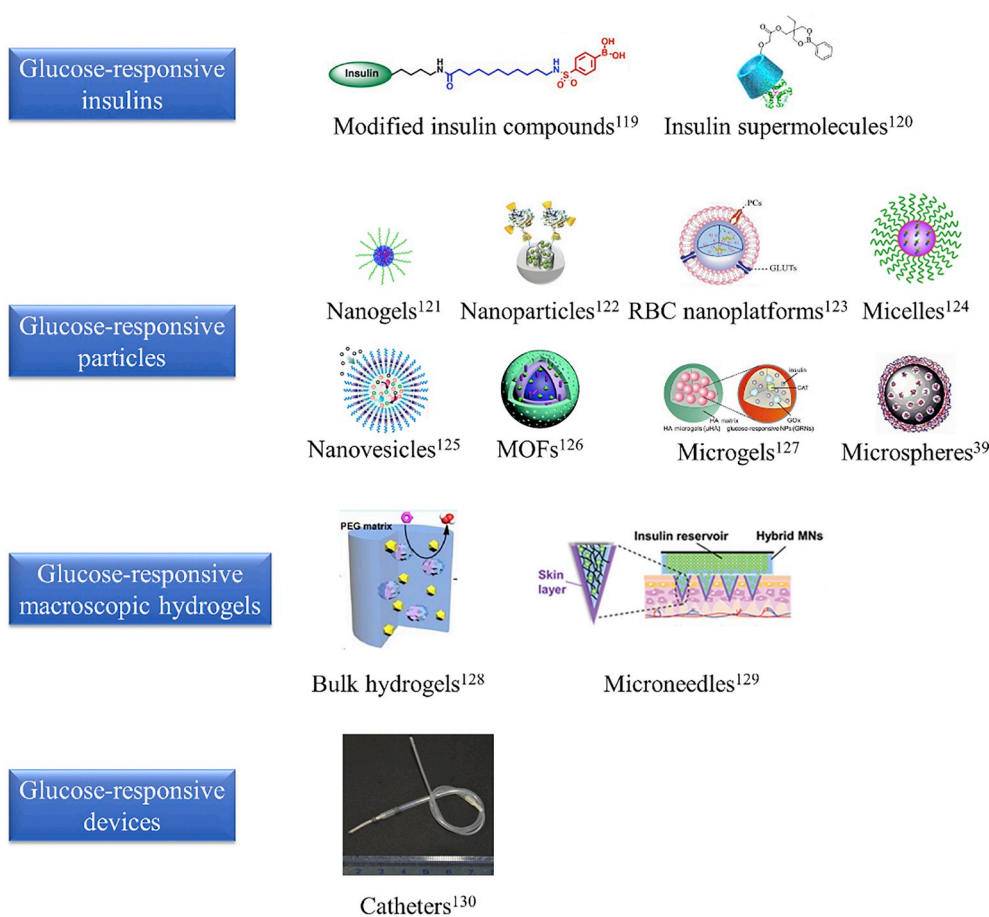


Fig. 16. The classification of glucose-responsive systems and their typical patterns including modified insulin compounds (Adapted with permission from ref. [119]. Copyright 2015 National Academy of Sciences.), insulin supramolecules (Adapted with permission from ref. [120]. Copyright 2018 Elsevier.), nanogels (Adapted with permission from ref. [121]. Copyright 2015 The Royal Society of Chemistry.), nanoparticles (Adapted with permission from ref. [122]. Copyright 2017 Wiley Online Library.), red-blood-cell (RBC) nanoplatfoms (Adapted with permission from ref. [123]. Copyright 2018 Wiley Online Library.), micelles (Adapted with permission from ref. [124]. Copyright 2018 American Chemical Society.), nanovesicles (Adapted with permission from ref. [125]. Copyright 2014 American Chemical Society.), MOFs (Adapted with permission from ref. [126]. Copyright 2018 The Royal Society of Chemistry.), microgels (Adapted with permission from ref. [127]. Copyright 2015 Springer.), microspheres (Adapted with permission from ref. [39]. Copyright 2017 Taylor & Francis.), macroscopic hydrogels (Adapted with permission from ref. [128]. Copyright 2017 American Chemical Society.), microneedles (Adapted with permission from ref. [129]. Copyright 2019 Wiley Online Library.) and catheters (Adapted with permission from ref. [130]. Copyright 2017 American Association for the Advancement of Science.). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Glucose-responsive insulin delivery systems

Based on the discussion of the two challenges, it is promising to fabricate smart insulin delivery systems with different strategies and the controllable insulin releasing rate. Herein, four groups of smart insulin delivery systems are defined according to the sizes including glucose-responsive molecules and supramolecules, glucose-responsive particles, glucose-responsive macroscopic hydrogels and glucose-responsive devices. Fig. 16 gives typical patterns of each group.

4.1. Glucose-responsive molecules and supramolecules

Insulin molecules [119] or insulin-containing supramolecules [120] could be modified by PBA and its derivatives to obtain glucose-responsive properties. Such a group of glucose-responsive insulin of the nano- or subnano-scale could be simply delivered into the human circulative system by injection [119].

Chou et al. [119] synthesized a series of chemical modified insulins covalently bonded with aliphatic chains and PBA moieties as shown in Fig. 17. The aliphatic domains, inspired by the long-acting insulin detemir, were designed to prolong the BGLs-controlling period, while the PBA moieties provided the modified insulin molecules with glucose responding behaviors [119]. The modified insulins showed improved BGLs-controlling performance, and the longest effecting time could be extended to more than 3 times in comparison with those without PBA moieties [119].

Glucose-responsive supramolecules could be designed based on the host-guest chemistry. For example, Seki et al. [131] prepared a host-guest inclusion complex containing γ -cyclodextrin and insulin. Glucose-responsive γ -cyclodextrin was modified by nitrophenylboronic acid

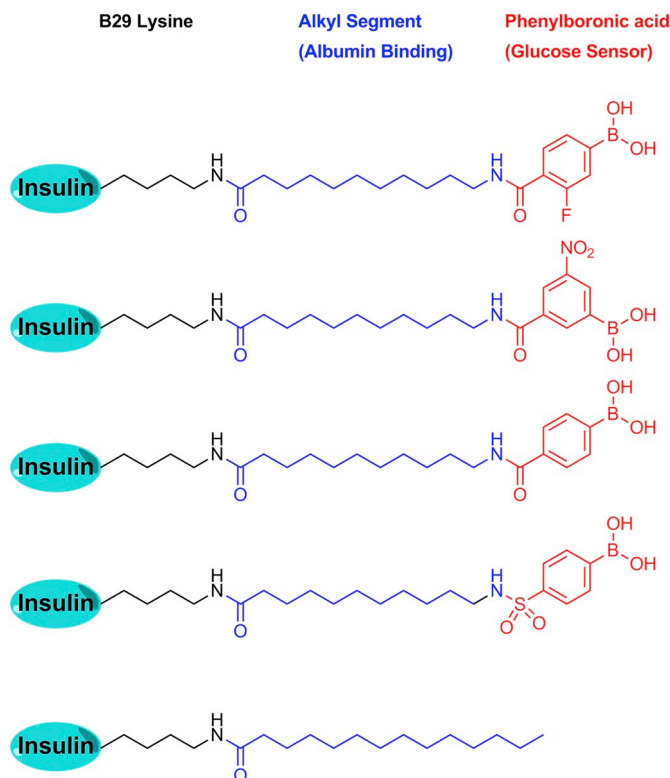


Fig. 17. The structures of aliphatic PBA-modified insulins and the aliphatic insulin [119].

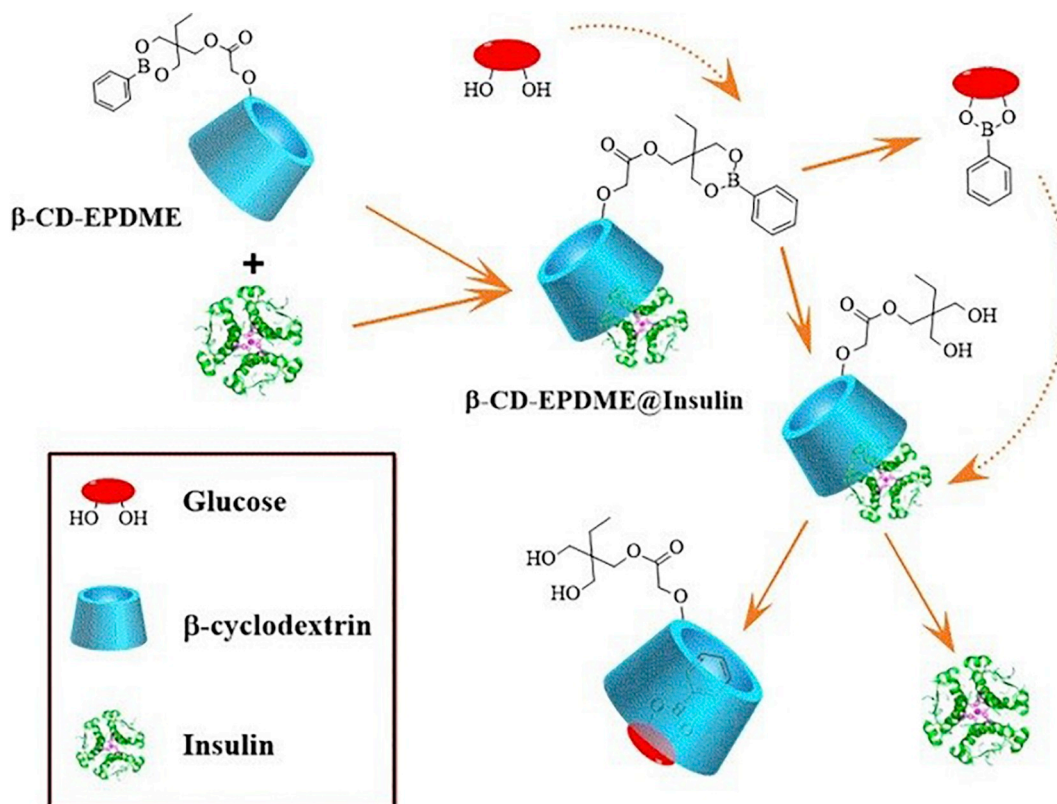


Fig. 18. The glucose-responsive mechanism of insulin/ β -cyclodextrin supramolecules. (Adapted with permission from ref. [120]. Copyright 2018 Elsevier.)

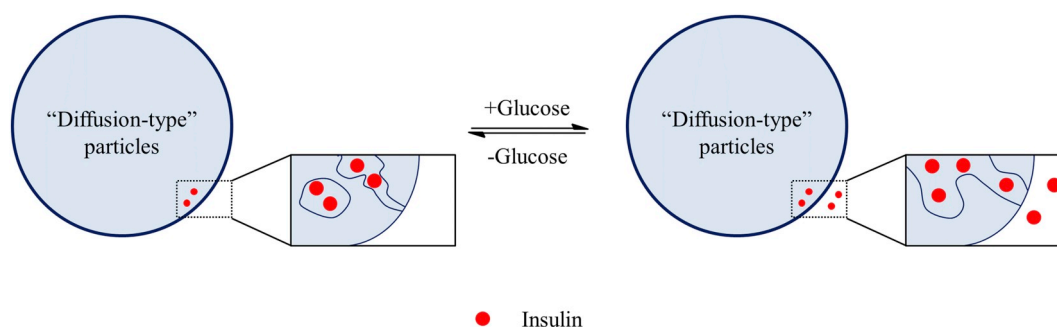


Fig. 19. The glucose-responsive mechanism of “diffusion-type” particles [122].

(NPBA) while insulin was modified by naphthalene-terminated PEG. The modified insulin could be released out due to the bonding of NPBA moieties and glucose [131]. Fig. 18 gave the other example that Xu et al. [120] fabricated a host-guest inclusion complex based on PBA-grafted cyclodextrin and unmodified insulin. Both of these two systems aimed to regulate the leaving behaviors of insulin by changing the hydrophilicity of modified cyclodextrins.

4.2. Glucose-responsive particles

From the nm to μm scale, particles loaded with both insulin and glucose-responsive units are also designed to prolong the BGLs-controlling period in the injection administration [123]. With acid- and enzyme-resistant treatments, the glucose-responsive insulin-loaded particles could also be used in the oral administration [46]. According to the insulin releasing mechanism, these particles are classified into diffusion type and click type.

4.2.1. “Diffusion-type” particles

In a “diffusion-type” particle, the insulin carrier acts as a regulator

when insulin transfers from the inner channels of the particle to the outer environments [122]. As shown in Fig. 19, the inner channels turn wider to promote the insulin releasing rate under high BGLs and return to the normal sizes under normal BGLs [122]. This type of particles usually shows a stable decline for the insulin releasing rate due to the gradual loss of insulin inside the particles [122].

Polymeric nanoparticles or microparticles are typical “diffusion-type” particles. For example, Chang et al. [132] designed insulin-loaded nanoparticles assembled by Con A and amylopectin. The Con A/amylopectin interaction could be weakened under the increasing glucose concentration [132]. However, insulin-loaded nanoparticles or microparticles usually possess “burst effect” which results in uncontrollable initial drug releasing behaviors [133–135]. In order to solve this problem, the particles could be designed as core-shell structures [136]. Wu et al. [39] adopted the layer-by-layer technique to encapsulate insulin-loaded PLGA microspheres with PBA-containing polymers and poly(vinyl alcohol) (PVA). Thus, the “burst effect” in the first day was significantly avoided [39].

Functionalized silica nanoparticles are also studied for insulin loading due to the high loading capacity and biocompatible [137].

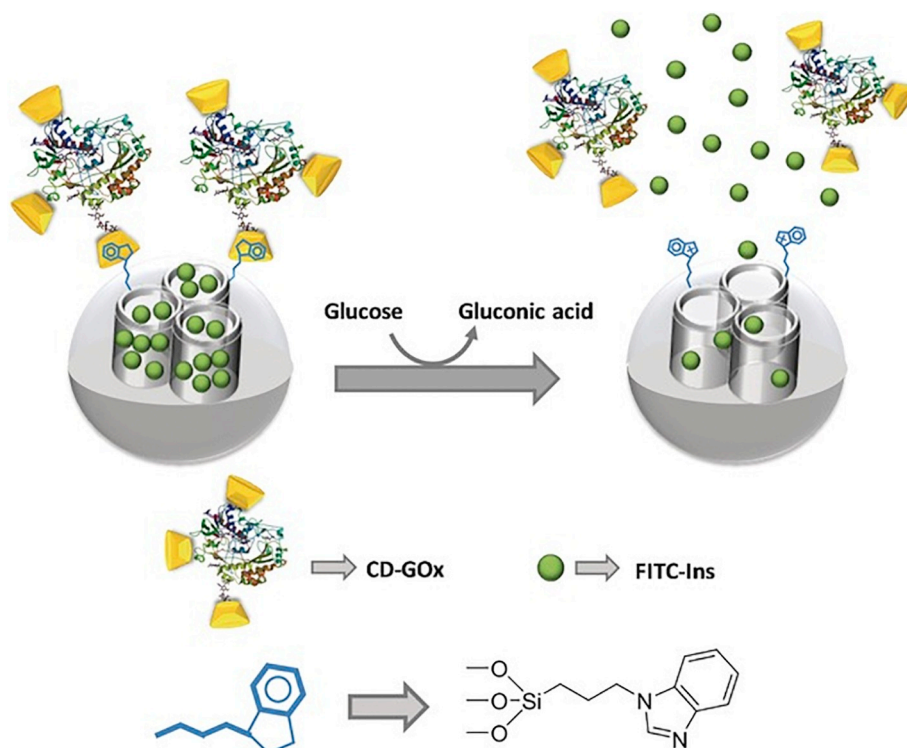


Fig. 20. The glucose-responsive mechanism of GOx-based silica nanoparticles. (Adapted with permission from ref. [122]. Copyright 2017 Wiley Online Library.)

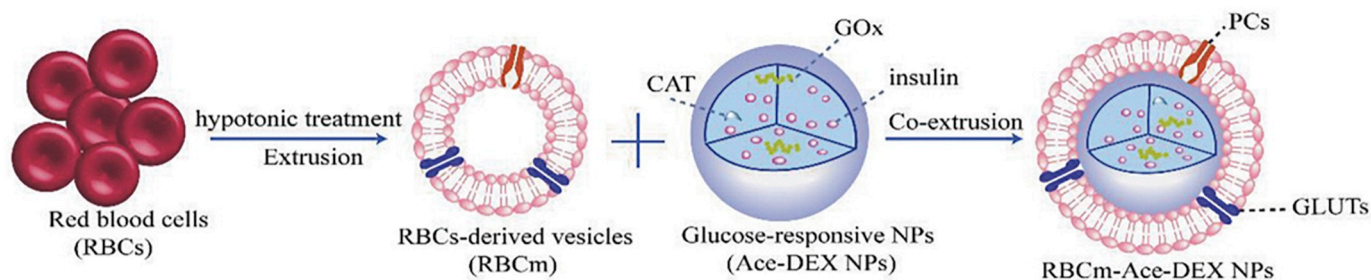


Fig. 21. Preparation of the RBC nanoplatforms for insulin delivery. (Adapted with permission from ref. [123]. Copyright 2018 Wiley Online Library.)

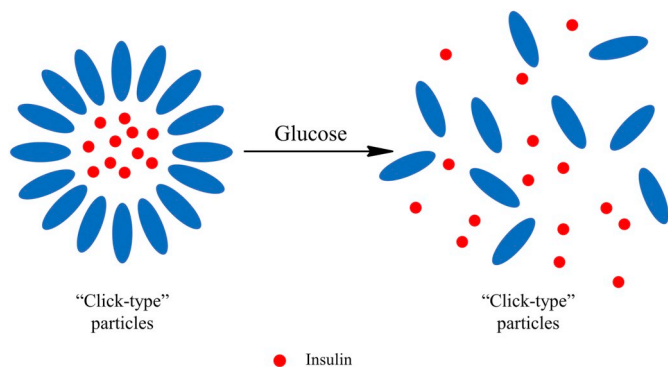


Fig. 22. The general mechanism of insulin release in "diffusion-type" particles [126].

Oroval et al. [122] designed a GOx-based silica nanoparticles capped by β -cyclodextrin/benzimidazole inclusion complexes as shown in Fig. 20. Insulin was loaded in the inner channels of these particles [122]. Therefore, under a high glucose concentration, GOx could provide an acidic environment which further promoted dissociation of the "caps" and the release of insulin [122]. Similarly, gold nanowires could be

modified for glucose-responsive insulin delivery as well [138].

Peptides with special segments possess could form coacervates which are deemed to be the potential carriers in drug delivery systems. Lim et al. [139] encapsulated insulin and GOx in the coacervate droplets of DgHBP, a kind of peptides separated from the Humboldt squid beak. The peptide coacervates were pH-sensitive, so that their dissociation as well as insulin releasing behaviors were triggered by GOx when the glucose concentration increased [139].

Glucose-responsive microgels and nanogels with three-dimension networks also act as "diffusion-type" particles due to the inner channels of tunable sizes. Typically, a portion of the crosslinking points are unstable and dissociable under a high glucose concentration, which induces the swelling ratios of the gels to increase and further promotes the insulin releasing behaviors. Herein, Con A-sugar-based crosslinking points [140] and boronic ester crosslinking points [121,141] are the most widely studied. An alternative approach to fabricate glucose-responsive gels is based on the glucose-induced transfer from hydrophobicity to hydrophilicity [142,143]. For example, Lee et al. [144] reported a kind of insulin-loaded nanogels where the PBA moieties in the gel networks obtained improved hydrophilicity at high glucose levels and insulin could be released out controllably.

Rapid immune clearance is a universal problem for drug-loading particles in the injection administration. As a solution, red blood cells

Table 5
The working mechanisms of different amphiphilic polymers.

Hydrophilic end	Hydrophobic end	Glucose-responsive material	Particle morphology	Mechanism of hydrophobic- hydrophilic transfer	Ref.
PEO	PBA moieties	PBA	Micelles	Formation of PBA/glucose complexes	[148]
PEG	PBA moieties	PBA	Micelles	Formation of PBA/glucose complexes	[150]
EG & OEG	PBA moieties	PBA	Micelles	Formation of PBA/glucose complexes	[151]
PEG	Coumarin moieties & PBA moieties	PBA	Micelles	Formation of PBA/glucose complexes	[146]
PEG	Coumarin moieties & PBA moieties	PBA	Micelles	Formation of PBA/glucose complexes	[124]
Sulfobetaine moieties	PBA moieties	PBA	Micelles	Formation of PBA/glucose complexes	[152]
PEG	Diisopropyl amino groups	GOx	Micelles	Protonation of tertiary amines	[149]
PEG	Ketal	GOx	Vesicles	Hydrolysis of ketals	[125]
Pillararene/ PBA inclusion complexes	Alkyl groups	PBA	Vesicles	Formation of PBA/glucose complexes	[153]
Gluconic acid moieties	PBA moieties	PBA	Nanoparticles	Formation of PBA/glucose complexes and dissociation of PBA/sugar moieties crosslinking points	[154]

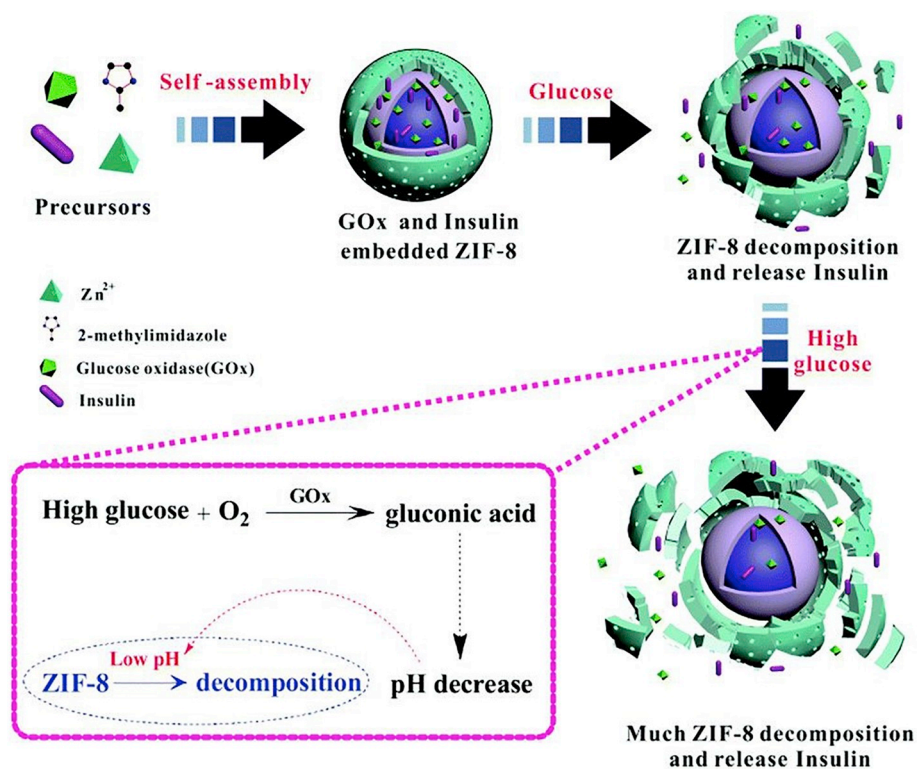


Fig. 23. The synthetic route and the glucose-responsive mechanism of the insulin-loaded Zn²⁺/imidazolate- based ZIFs. (Adapted with permission from ref. [126]. Copyright 2018 The Royal Society of Chemistry.)

(RBCs) were used as camouflage materials in injective insulin delivery systems [123,145]. In Fig. 21, Fu et al. [123] designed a GOx-based glucose- and pH-responsive system to control the insulin releasing behaviors, and this system was creatively encapsulated in the RBCs. Thus, the glucose-responsive insulin release was successful and immune clearance was avoided as well [123].

4.2.2. “Click-type” particles

“Click-type” particles are defined as self-assembled particles which dissociate quickly in glucose-containing solutions [146]. In comparison with “diffusion-type” particles, insulin loaded in the “click-type” particles is directly exposed instead of slowly diffusing into the outer environment under a high glucose concentration, so “click-type” particles usually possess a much faster insulin releasing rate [146]. Fig. 22 illustrates the general mechanism of insulin release in “click-type” particles [126].

PBA-modified polyelectrolyte microcapsules are typical “click-type” particles, in which layer-by-layer electrostatic interaction could be interfered under a high glucose concentration. Shi et al. [147] prepared the glucose-responsive polyelectrolyte capsules with two different polymers which contained PBA moieties and saccharide pendant groups respectively layer by layer. Insulin encapsulated inside could be released out when the boronic ester bonds between two adjacent layers dissociated under an increased glucose concentration [147].

Glucose-triggered amphiphilic polymers are also synthesized to fabricate micelles, vesicles and other “click-type” particles. PBA-modified polymers are widely studied in this field since the hydrophobic PBA moieties could convert into hydrophilic PBA/glucose complexes [148]. Moreover, GOx could also be applied. Li et al. [149] synthesized tertiary-amine-containing polymers based on the pH-responsive mechanism of GOx. GOx provided an acidic environment under a high glucose concentration and then enhanced the combination of tertiary-

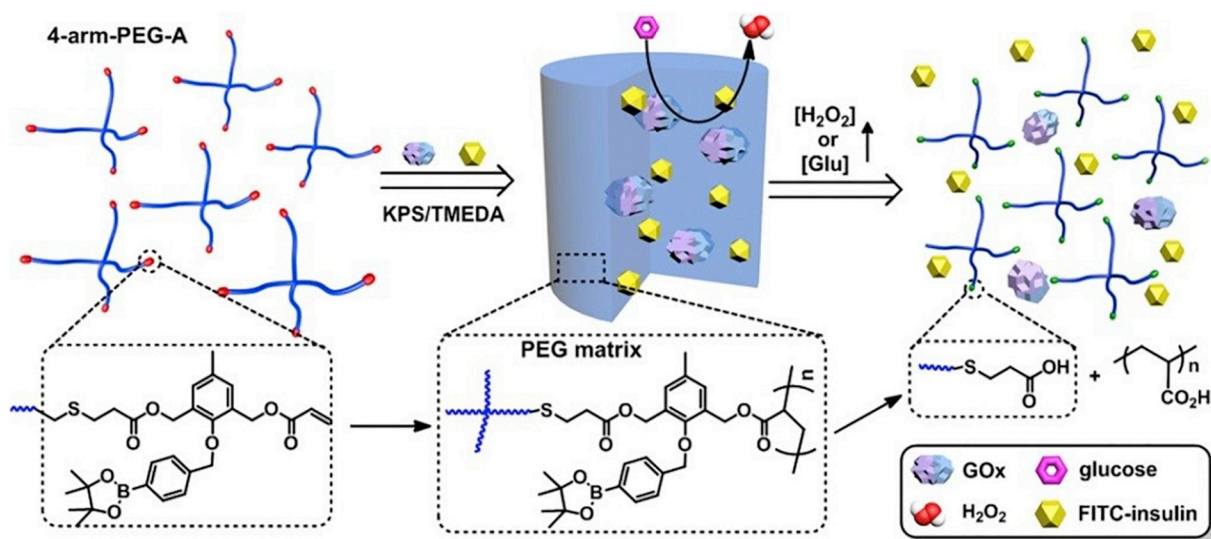


Fig. 24. The synthetic route and the dissociation mechanism of H₂O₂-sensitive insulin-loaded hydrogels. (Adapted with permission from ref. [128]. Copyright 2017 American Chemical Society.)

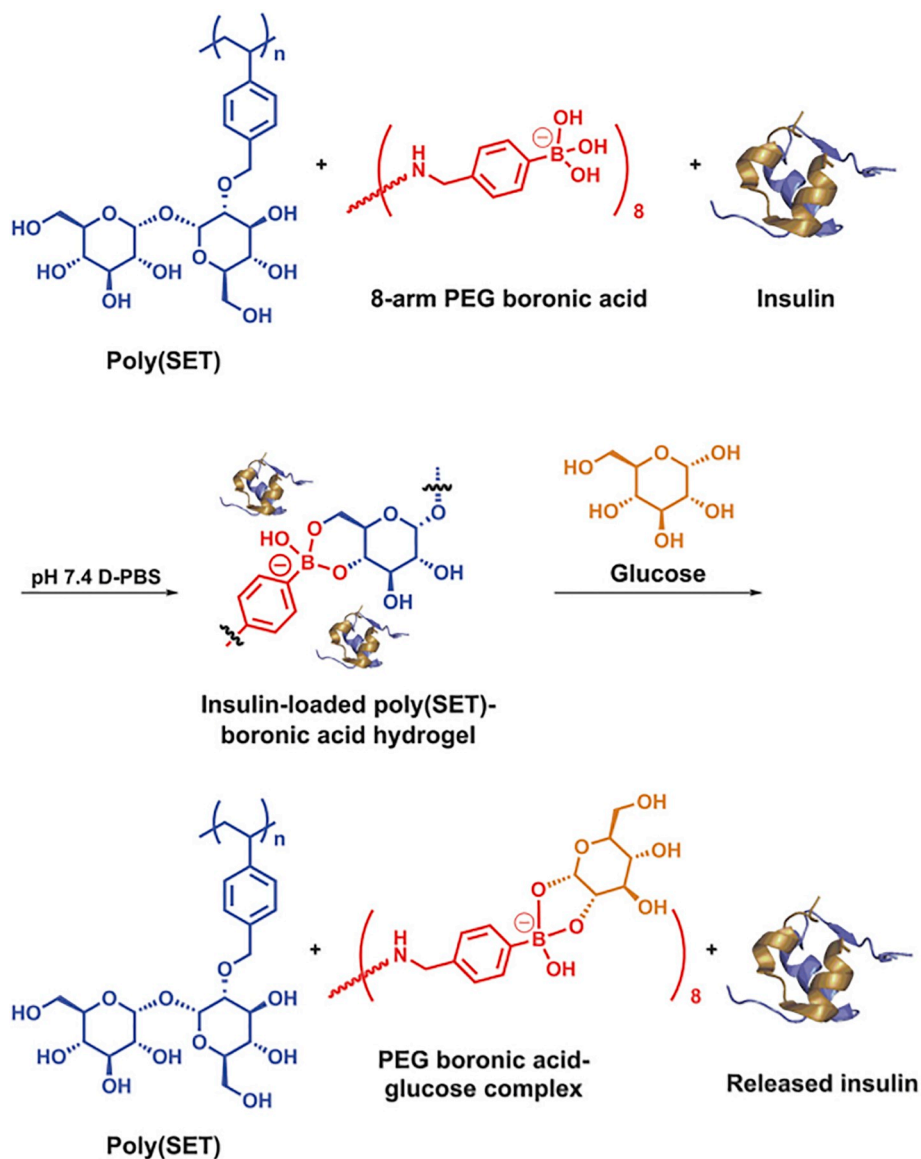


Fig. 25. Formation and dissociation of the injectable hydrogels with boronic ester crosslinking points. (Adapted with permission from ref. [161]. Copyright 2018 Wiley Online Library.)

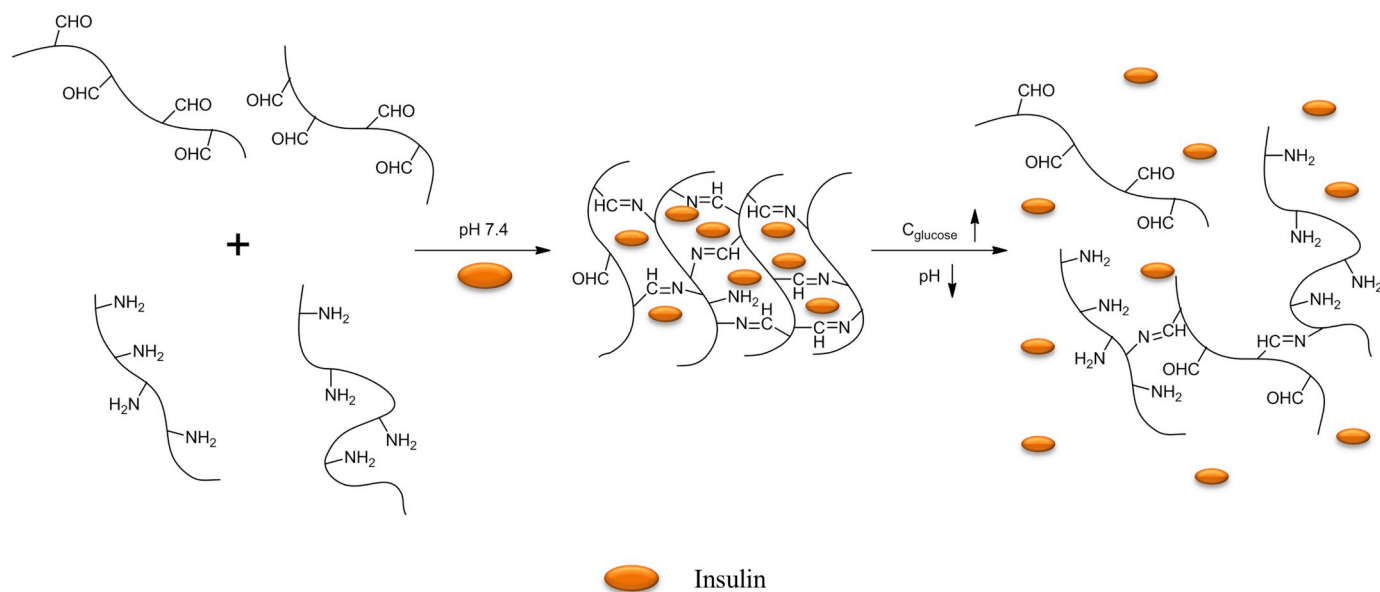


Fig. 26. The glucose-responsive mechanism of schiff-base-containing injectable hydrogels [41].

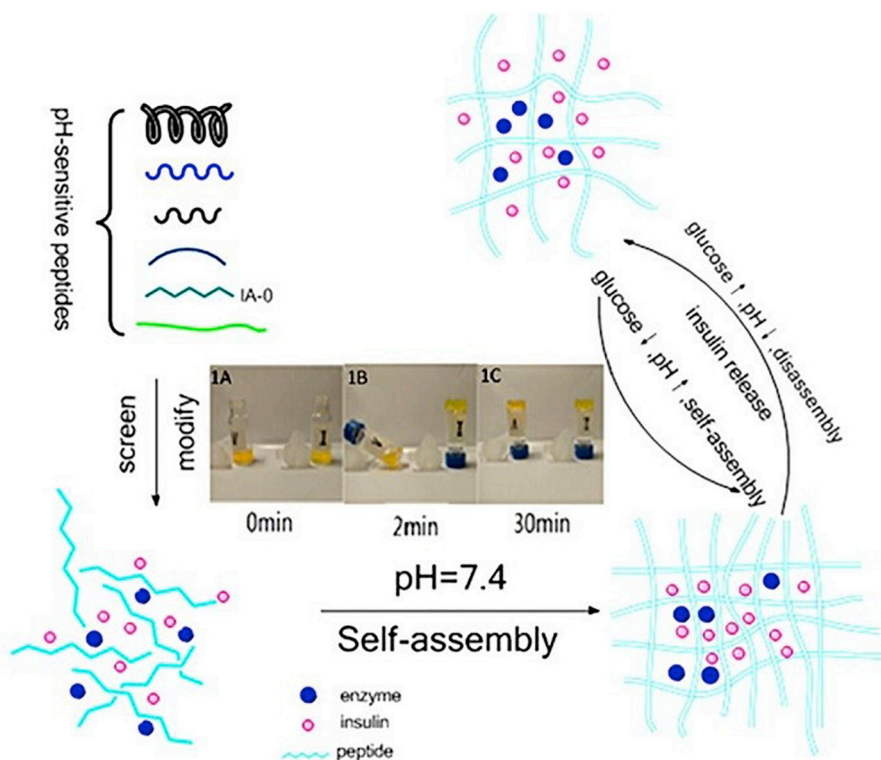


Fig. 27. The gelation process and the glucose-responsive mechanism of the peptide hydrogels. (Adapted with permission from ref. [163]. Copyright 2017 Elsevier.)

amino groups and protons. Protonated tertiary-amino groups led to dissolution of the initially amphiphilic chains, which promoted the insulin releasing behaviors [149]. Table 5 summarizes the components of different amphiphilic polymers as well as their working mechanisms.

MOFs could also be classified as “click-type” particles in acid media. Duan et al. [126] prepared insulin-loaded Zn^{2+} /imidazolate-based MOFs (also named as ZIFs) as shown in Fig. 23. GOx was added in the system as a trigger to create an acidic environment and further make the particles dissociate at high glucose levels.

Contrary to the RBC-based “diffusion-type” particles above, Xia et al. [155] designed another RBC-camouflaged insulin delivery system of ultrafast response. In this case, GOx was creatively grafted on the

surface of RBC membranes, so that H_2O_2 generated under a high glucose concentration could destroy the RBC membranes in a short time and insulin could be released out quickly [155].

Self-regulated nanoparticles fabricated by acetal-modified dextran and GOx were also reported as the “click-type” particles [156]. The acid-sensitive acetal-modified dextran transferred into soluble dextran under a high glucose concentration [156]. Thus, the encapsulated insulin could be released out [156].

4.3. Glucose-responsive macroscopic hydrogels

Hydrogels of the mm scale or even larger sizes act as insulin-loaded

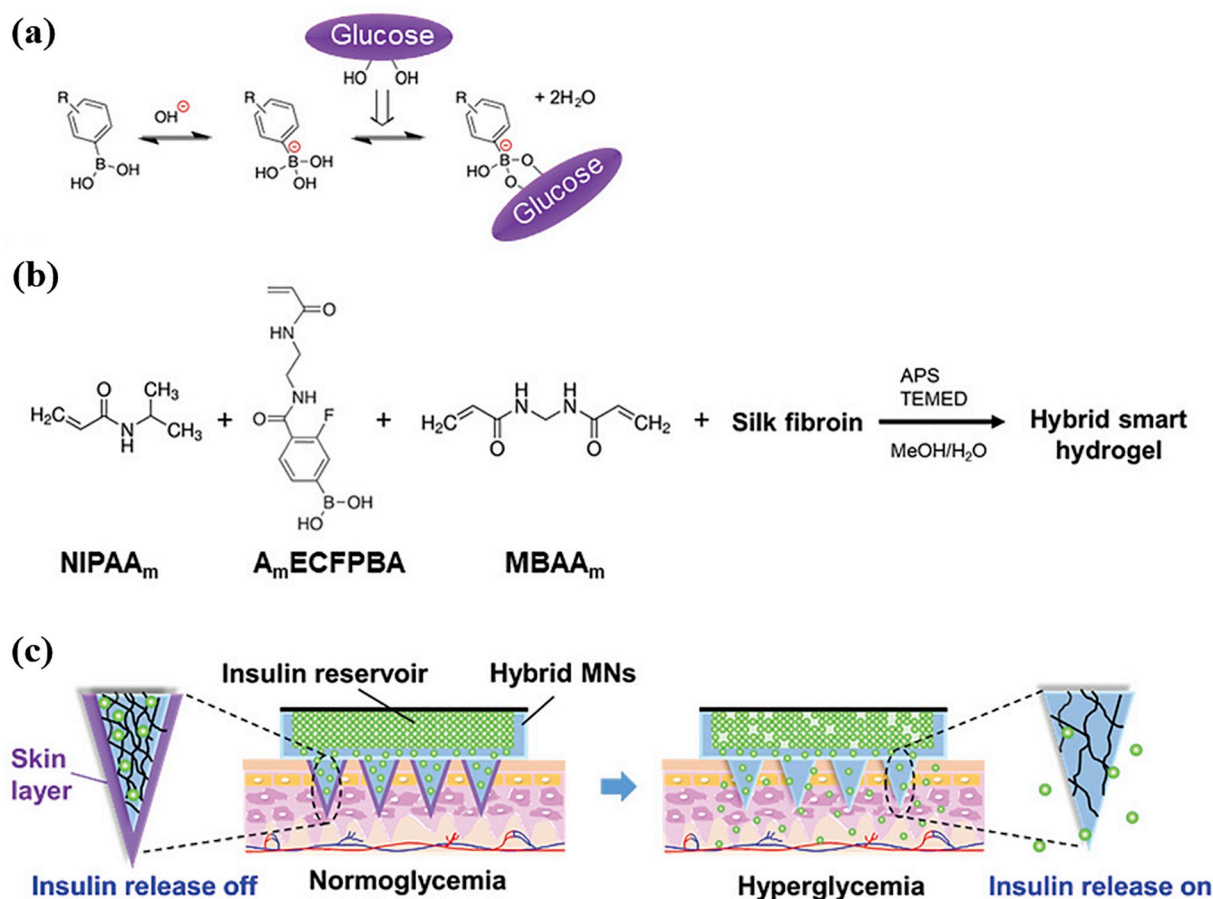


Fig. 28. (a) The general glucose-responsive equilibria of PBA and its derivatives. (b) Preparation of the hybrid hydrogel. (c) The schematic diagram of hybrid microneedles and the insulin releasing mechanism depending on BGLs. (Adapted with permission from ref [129]. Copyright 2019 Wiley Online Library.)

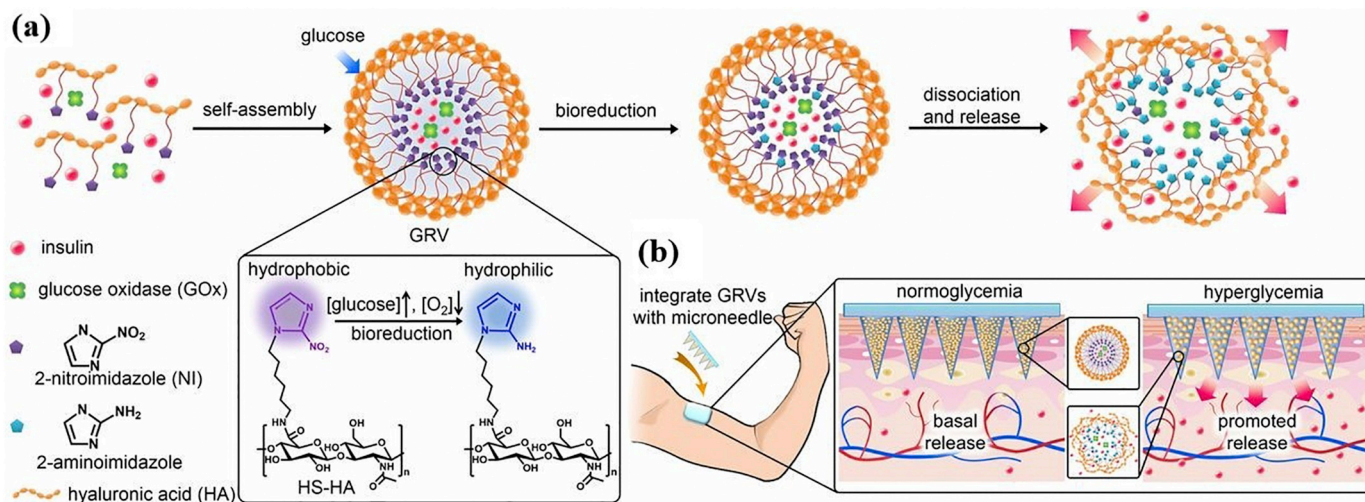


Fig. 29. The glucose-responsive MNPs based on the hypoxia-responsive mechanism of GOx. (a) Formation and dissociation of the glucose-responsive vesicles (GRVs). (b) The MNPs containing GRVs for insulin delivery and their different insulin releasing behaviors at normoglycemia and hyperglycemia. (Adapted with permission from ref [43]. Copyright 2015 National Academy of Sciences.)

platforms with more complex structures in comparison with molecules and particles. The insulin releasing rates of the glucose-responsive macroscopic hydrogels depends on the tunable sizes of the inner channels which are typically regulated by changing hydrophilicity of the hydrogel networks [65] or breaking the crosslinking points [157]. Potential applications of these hydrogels are related to the injection administration [158], transdermal administration [159] and oral

administration [160] of insulin.

4.3.1. Bulk hydrogels

Bulk hydrogels of three-dimensional networks usually possess definitive morphologies and protect insulin against external effects such as acid, enzymes, heat, etc [157,161]. Moreover, insulin diffuses out of the hydrogels with different rates which could be directly controlled by the

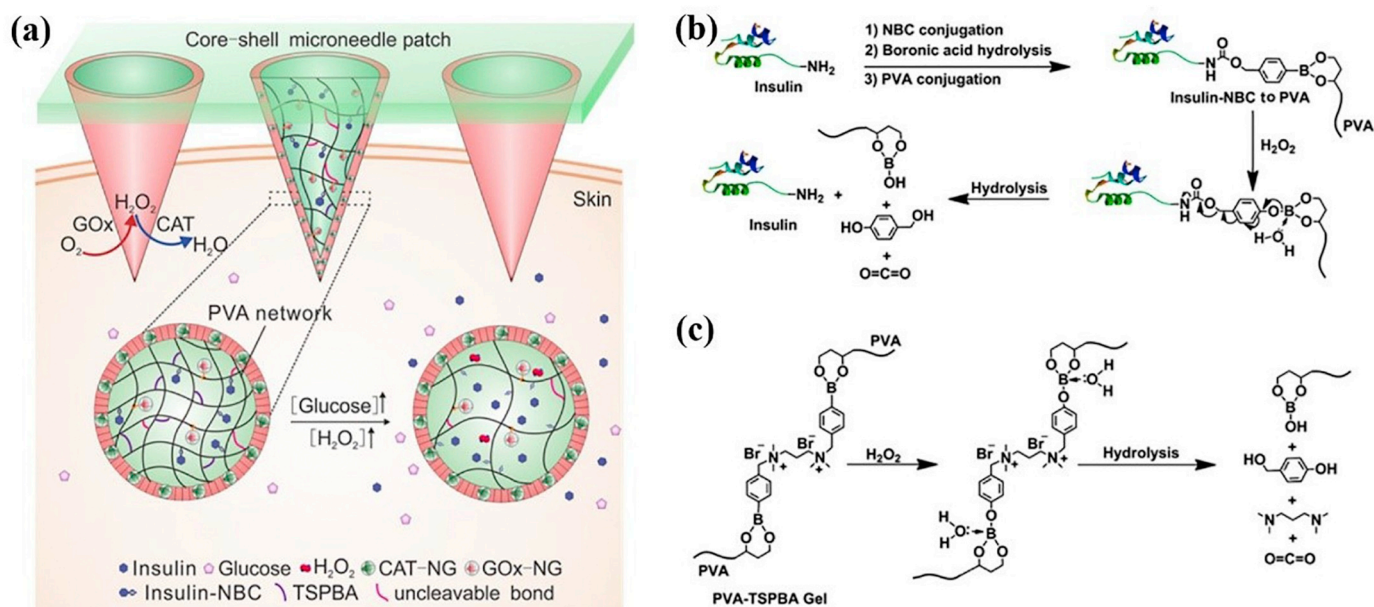


Fig. 30. The schematic diagram of the core-shell glucose-responsive MNPs for insulin delivery. (a) Insulin is triggered to release from the core part of the microneedles at hyperglycemia and excess H_2O_2 is consumed in the shell part by CAT. (b) Modification of insulin and its mechanism of H_2O_2 -triggered release. (c) The mechanism to break the boronic ester crosslinking points by H_2O_2 . (Adapted with permission from ref. [99]. Copyright 2018 American Chemical Society.)

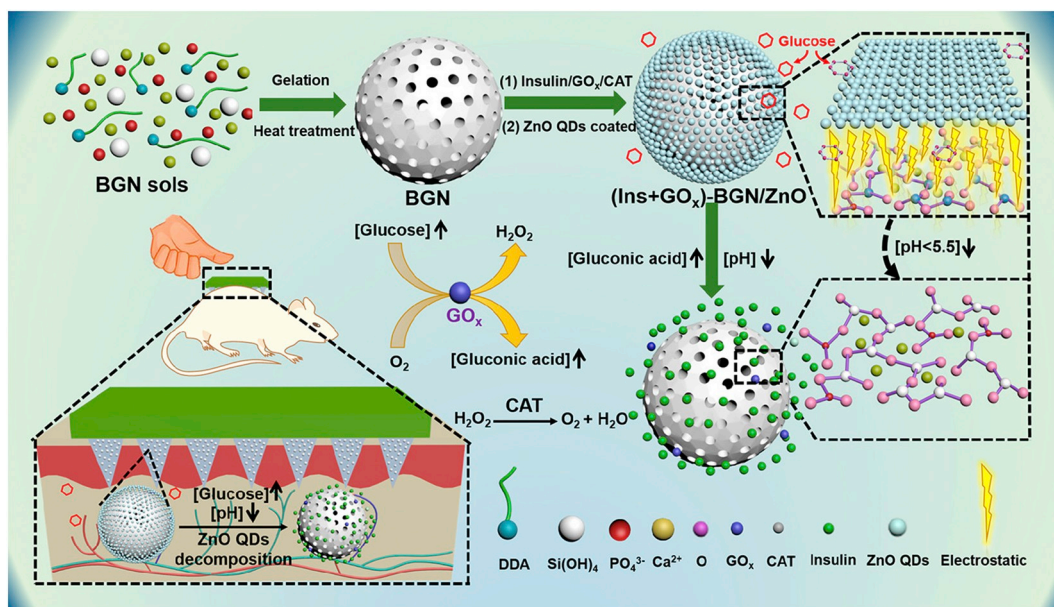


Fig. 31. Preparation of glucose-responsive microneedles integrated with ZnO quantum-dot-capped MBGs for transdermal delivery of insulin. (Adapted with permission from ref. [164]. Copyright 2018 American Chemical Society.)

tunable sizes of the inner channels under different glucose concentrations [15].

It is a feasible approach to control the insulin releasing behaviors by modifying the hydrogels with PBA moieties [65]. An increased glucose concentration could promote hydrophilicity of PBA moieties and improve the swelling ability of the hydrogels which result in the enlarged sizes of their inner channels [65]. In contrast, hydrogels with PBA/diol crosslinking points follow a totally different working mechanism [12]. Here the boronic ester bonds could be broken by the competitive reaction with glucose or oxidation by GOx [12]. The decreased crosslinking points promote the insulin releasing rate [12]. Zhang et al. [128] prepared the insulin/GOx-coencapsulated hydrogels with PBA-pinacol-ester-modified crosslinking points as shown in Fig. 24. These crosslinking points could be broken by H_2O_2 , thus the insulin releasing

rate could be promoted under a high glucose concentration [128].

Bulk hydrogels are extremely suitable for insulin delivery via oral administration since they could protect insulin against acid and enzymes [157,161]. For example, Li et al. [162] dispersed insulin-loaded nanocarriers (INCs) with PBA moieties in the crosslinked hyaluronic acid (HA) hydrogels to avoid insulin denaturation [162]. Compared with that without HA gels, the INC/HA system contained more insulin with bioactivity when going through the stomach [162].

4.3.2. Injectable hydrogels

Injectable hydrogels, in comparison with the bulk hydrogels, are usually crosslinked by weak chemical bonds which could form under mild conditions [13]. Thus, bioactivity of insulin could be well kept in the fabrication of injectable hydrogels.

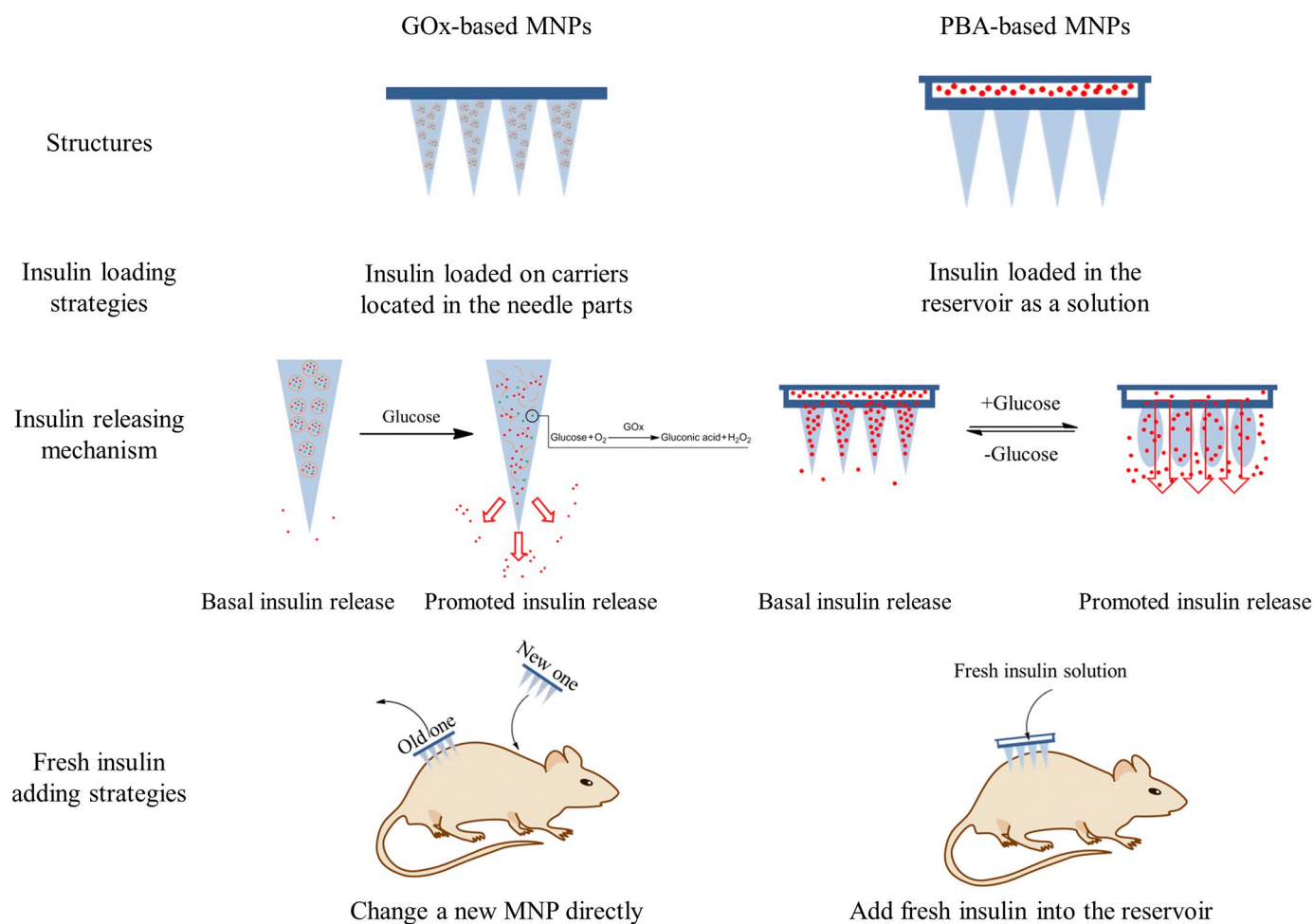


Fig. 32. Comparative data between the GOx-based microneedles and the PBA-based microneedles [43,129].

The boronic ester bonds are reversible, of which dissociation could be triggered by glucose [78]. The gelation process of the injectable hydrogel with this type of crosslinking points could occur by simply mixing the PBA moieties-containing solution and saccharide moieties-containing solution [161]. In Fig. 25, Lee et al. [161] reported the injectable hydrogels fabricated by trehalose-grafted polymers and PBA-modified PEG. Moreover, the dynamic boronic ester bonds could be formed within 5 min [161].

The pH-triggered strategy for insulin delivery, which is based on the interaction between glucose and GOx, is an alternative approach to prepare injectable hydrogels. Schiff bases are the promising candidates as the pH-sensitive crosslinking points of this mechanism (Fig. 26), because such kind of injectable hydrogels could be easily formed at pH 7.4 and be dissolved in the acidified solution [41].

Besides the covalent bonds, non-covalent interactions, such as hydrogen bonding, electrostatic interaction and hydrophobic interaction, also could promote the gelation process [163]. For instance, Li et al. [163] prepared injectable hydrogels with acid-sensitive peptides as shown in Fig. 27. Under an increased glucose concentration, the pH value was reduced via GOx, which promoted dissolution of the hydrogels and the insulin releasing rate [163].

4.3.3. Hydrogel-based microneedle patches

Hydrogel-based microneedle patches loaded with glucose-responsive units are emerging technologies for smart insulin delivery [28]. To some extent, the hydrogel-based microneedles are similar to the bulk hydrogels because mechanical strength provided by stable crosslinking points is necessary for microneedles to insert into human

skins [12]. Additionally, glucose-responsive particles are also introduced into MNPs to form hybrid platforms for enhanced stability of BGLs [43].

Hydrogels containing PBA moieties show potentials in fabricating microneedles of high stability. Chen et al. [129] reported the first PBA-based MNPs for insulin delivery. As shown in Fig. 28, AmECFPBA ($pK_a = 7.2$) and NIPAAm were adopted to prepare the microneedles with glucose-responsive behaviors under physiological conditions [129]. The semi-interpenetrating network formed by PBA-based hydrogels and silk fibroin enormously improved the mechanism strength of microneedles [129]. Moreover, an insulin reservoir was designed for the long-term usage, so that the fresh insulin solution could be simply added [129]. Insulin diffused through the inner channels of microneedles and then permeate into human bodies [129].

In comparison with PBA-based microneedles, more glucose-responsive MNPs are designed based on GOx. For instance, Yu et al. [43] reported a GOx-based MNP of the hypoxia-responsive mechanism (Fig. 29). Insulin and GOx were preliminarily encapsulated in self-assembled polymeric vesicles (PVs) which were dispersed in the needle part of a MNP [43]. 2-nitroimidazole (NI) groups were grafted on the polymer chains of the PVs as the hypoxia-sensitive triggers [43]. Under high BGLs, GOx could rapidly consume dissolved oxygen to construct a hypoxic microenvironment which resulted in dissociation of PVs and the release of insulin [43]. However, by-product H_2O_2 remained in the MNP and even diffused into tissues which could result in inflammation [43].

To solve this problem, Wang et al. [99] designed a core-shell H_2O_2 -responsive MNP with H_2O_2 -scavenging performances as shown in

Table 6
The glucose-responsive MNPs applied on diabetic mice.

Material	Fabrication method	Response target	Structure		Insulin type		Dose IU/kg	C _{max} μU/mL	T _{max} h	RBA %	C _{min}		T _{min}		RPA %	Ref.
			D _h /μm	D _v /μm	H/μm							%	h	h		
HA	Self-assembly + molding	Hypoxia	150	10	600	Human recombinant insulin (27.5 IU/mg of Zn salt)	275	1120	1.5	-	33	1	-	-	-	[43]
HA	Self-assembly+molding	Hypoxia	400	5	800	Pancreatic β-cell	-	-	-	-	40	4	-	-	-	[165]
PVA	Nanogel encapsulation + molding	H ₂ O ₂	300	5	600	Human recombinant insulin	1375	2200	1	-	23	2	-	-	-	[99]
HA	Self-assembly+molding	H ₂ O ₂	-	-	-	Human recombinant insulin (27.5 IU/mg of Zn salt)	275	1500	1	-	15	1.5	-	-	-	[166]
PVP	MNSs encapsulation + molding	H ₂ O ₂	250	-	550	Porcine insulin (30 IU/mg)	40	350	3	92.6	20	3	90.1	-	-	[167]
PVP	MBGs encapsulation + molding	pH	250	20	550	Porcine insulin (30 IU/mg, from porcine pancreas)	20	290	2	96.2 ± 3.6	36	2	95.3 ± 2.8	-	-	[164]
PVP + PVA	Self-assembly+molding	Glucose + H ₂ O ₂	250	-	500	Porcine insulin (30 IU/mg)	40	-	2	-	-	4	-	-	-	[168]
HA	Self-assembly+molding	Hypoxia + H ₂ O ₂	300	10	600	Human recombinant insulin	275	1300	2	-	29	3	-	-	-	[169]
PVA	Self-assembly+molding	H ₂ O ₂ + pH	300	5	600	Human recombinant insulin	-	1150	1	-	22	1	-	-	-	[170]

Fig. 30. The core parts of the microneedles were partially crosslinked by H₂O₂-sensitive boronic esters while H₂O₂ could be generated under high BGLs in the enzymatic reaction of GOx [99]. Therefore, these boronic ester crosslinking points were broke by H₂O₂, which promoted insulin to release out [99]. Meanwhile, catalase (CAT), as the H₂O₂ scavenger, was loaded in the shell parts of the microneedles to avoid inflammation [99].

The pH-responsive mechanism was also realized on the organic/inorganic hybrid MNPs reported by Xu and his coworkers as shown in Fig. 31 [164]. Insulin, GOx and CAT were originally loaded in the inner channels of the mesoporous bioactive glasses (MBGs) [164]. Then MBGs were capped with acid-sensitive ZnO quantum dots (ZnO QDs) and dispersed in the microneedles [164]. GOx provided an acidic micro- environment under hyperglycemia conditions to dissolve the ZnO QDs [164]. Thus, insulin was subsequently promoted to be released out form MBGs. Such an organic/ inorganic hybrid MNP also possessed good mechanical strength [164].

Fig. 32 gives comparative data between the GOx-based microneedles and the PBA-based ones [43,129]. **Table 6** summarizes the glucose-responsive MNPs applied on diabetic mice with the materials, preparation methods and test results *in vivo*. Remarkably, all the GOx-based MNPs are combined with glucose-responsive particles while the hydrogel matrix of MNPs only acts as the transdermal carrier without glucose-responsive properties.

4.4. Glucose-responsive devices

Glucose-responsive devices over the mm scale, especially the artificial pancreas, are usually characterized as complex structures and long-term self-regulated insulin releasing behaviors [171–173]. The technologies for fabricating glucose-responsive particles and macroscopic hydrogels are also applied in the glucose-responsive devices [58,174,175].

Sahota et al. [176] studied an implantable device for controllable insulin delivery in a long term. The Con A/dextran complexes, as the glucose-responsive “on-off” switches, realized the stable insulin release for over 700 days [176].

Chu et al. [177] fabricated a different close-looped system based on GOx. Acid-sensitive NIPAAm/MAA nanoparticles were initially introduced into the outlet of the system in order to avoid the leakage of insulin [177]. These nanoparticles shrunk under high BGLs and swelled when the BGLs decreased, so that the insulin releasing rates could be controlled recurrently [177].

To avoid inactivation of GOx and cytotoxicity of Con A, Matsumoto et al. [130] designed a PBA-based device as an artificial pancreas (Fig. 33). The self-regulated insulin delivery under physiological conditions (pH 7.4, 37 °C) was realized *via* copolymerization of *N*-isopropylmethacrylamide (NIPMAAm), the temperature- sensitive component, and AmECFPBA, the glucose-sensitive component [130]. Herein, AmECFPBA possessed a pK_a value of 7.2, which was near from the physiological pH, and provided a sensitive response to the glucose concentration [130]. Meanwhile, NIPMAAm also provided the device with a sensitive response at the physiological temperature [130]. This device could automatically regulate the insulin releasing rates in many cycles according to the BGLs [130].

The polymeric film is another type of carrier to store and deliver insulin. Liang et al. [178] reported a honeycomb-patterned film to capture insulin in the cavities. The cavities were modified by 3-aminophenylboronic acid and alginate successively so that insulin aggregates could be immobilized on the surface of the cavities *via* electrostatic interaction (Fig. 34). When the glucose concentration increased, insulin could leave the surface of cavities under the competition of glucose and alginate for bonding with PBA moieties [178]. The insulin-capturing films possessed high releasing efficiency at hyperglycemia as well as stable storage properties at normoglycemia [178].

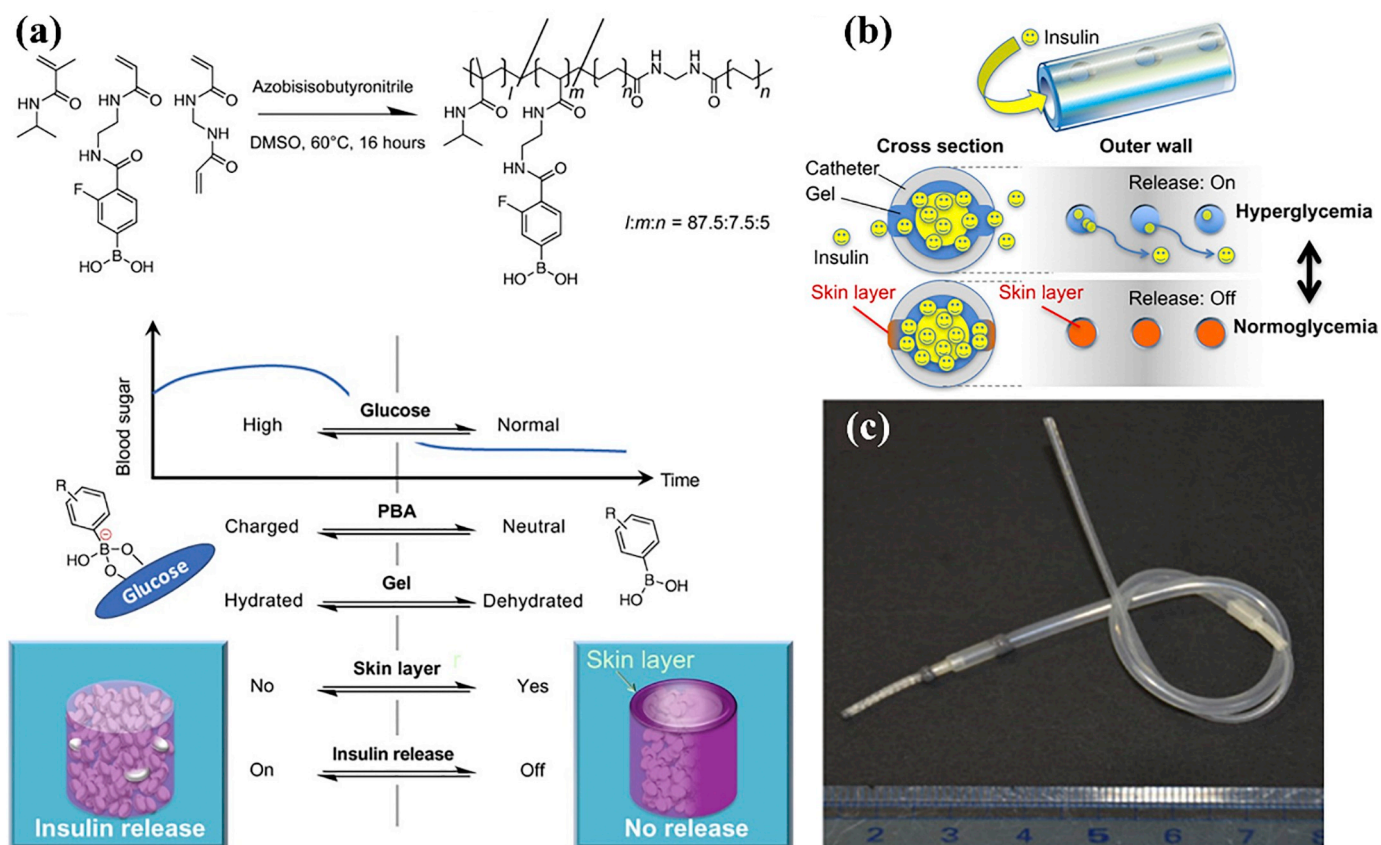


Fig. 33. (a) The preparing process and glucose-responsive mechanism of AmECFPBA/NIPMAAm-based hydrogels. (b) The insulin releasing behaviors of the artificial pancreas-like device at hyperglycemia and normoglycemia. (c) The photograph of the artificial pancreas-like device. (Adapted with permission from ref. [130]. Copyright 2017 American Association for the Advancement of Science.)

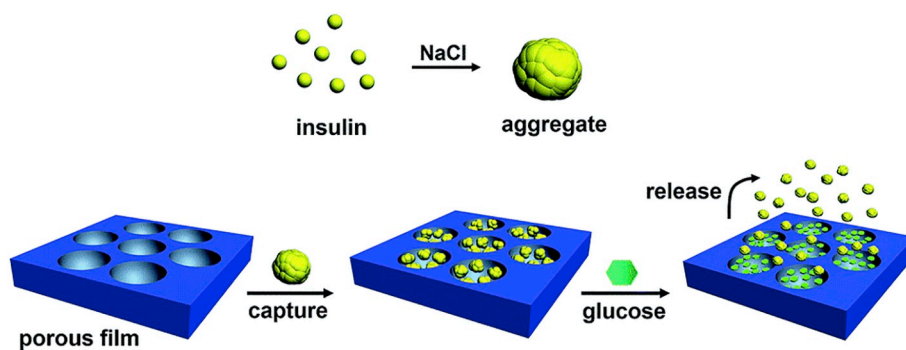


Fig. 34. Preparation of insulin aggregates and the insulin capturing and releasing behaviors based on the porous film. (Adapted with permission from ref. [178]. Copyright 2015 The Royal Society of Chemistry.)

5. Summary and outlook

Glucose-responsive insulin delivery possesses great potentials in diabetes care and overwhelms traditional insulin delivery *via* manual subcutaneous injection. Scientists devote themselves to create smart insulin delivery systems of controllable releasing rates. A series of products and devices with different sizes are fabricated for not only improved injectable insulin delivery systems but also oral, nasal and pulmonary approaches. The emerging technologies for smart insulin delivery are all based on different glucose-responsive strategies, materials and synthetic routes.

Apart from the advantages, several problems remain to be solved. Firstly, the insulin releasing behaviors, especially for the insulin delivery systems of high loading capacity, still have potentials to be explored. Since the insulin releasing rate is directly controlled by BGLs

and the insulin concentration in the delivery system, the releasing doses of insulin after meals show a continuous decline. Hence, it is necessary to avoid the possibilities of hypoglycemia in the several early doses and the insufficient insulin supply when loaded insulin is nearly exhausted. For the purpose of hypoglycemia prevention, GhavamiNejad et al. [179] reported the PBA-based microneedle patch for glucose-responsive glucagon delivery. Glucagon delivery was enhanced to raise BGLs at hypoglycemia [179]. Another promising solution is associated with the binary-drug loaded systems of insulin and glucagon. The alternative drug-releasing behaviors could be controlled by the glucose-responsive carrier matrix [180]. Moreover, to avoid the insufficient insulin supply, simulation of the “two-pool” mechanism for endogenous insulin secretion is worthy of consideration. Fresh insulin should be periodically added into the insulin delivery systems *via* external manipulation or self-regulated materials [129].

Secondly, the balance between the insulin administration frequency and the capacity of insulin in the delivery systems should be deeply studied and further achieved. A low frequency of insulin administration is the key to avoid pain, wounds and possible infection, but the low frequency correspondingly results in an increased dose of insulin with latent risks on the cells in the administration sites. One solution is to improve bioavailability of insulin. For the oral insulin delivery systems, the combination of glucose-responsive materials and cell-permeability enhancing agents could improve the bioavailability of insulin and reduce the drug-loading capacity [181,182].

Thirdly, bio-safety of glucose-responsive insulin delivery systems should be carefully considered. Residual monomers after polymerization and soluble molecules or oligomers in biodegradation of polymeric systems are delivered into human bodies together with insulin. These compounds possess ambiguous toxicological properties. Further evidences are needed to prove safety of the smart insulin delivery systems in the long-term usage. Systematic studies on the metabolic mechanisms of the monomers, polymers and degradation compounds should also be carried out.

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References

- N.H. Cho, J.E. Shaw, S. Karuranga, Y. Huang, J.D.D.R. Fernandes, A.W. Ohlrogge, B. Malanda, IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045, *Diabetes Res. Clin. Pract.* 138 (2018) 271.
- J. Tuomilehto, J. Lindström, J.G. Eriksson, T.T. Valle, H. Hämäläinen, P. Ilanne-Parikka, S. Keinänen-Kiukaanniemi, M. Laakso, A. Louheranta, M. Rastas, Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance, *N. Engl. J. Med.* 344 (2001) 1343–1350.
- M.R. Prausnitz, R. Langer, Transdermal drug delivery, *Nat. Biotechnol.* 26 (2008) 1261.
- J. Weissberg-Benchell, J. Antisdel-Lomaglio, R. Seshadri, Insulin pump therapy: a meta-analysis, *Diabetes Care* 26 (2003) 1079–1087.
- H.-C. Yeh, T.T. Brown, N. Maruthur, P. Ranasinghe, Z. Berger, Y.D. Suh, L.M. Wilson, E.B. Haberl, J. Brick, E.B. Bass, Comparative effectiveness and safety of methods of insulin delivery and glucose monitoring for diabetes mellitus: a systematic review and meta-analysis, *Ann. Intern. Med.* 157 (2012) 336–347.
- E. Cengiz, J.L. Sherr, S.A. Weinzimer, W.V. Tamborlane, New-generation diabetes management: glucose sensor-augmented insulin pump therapy, *Expert Rev. Med. Devices* 8 (2011) 449–458.
- R.M. Bergenstal, W.V. Tamborlane, A. Ahmann, J.B. Buse, G. Dailey, S.N. Davis, C. Joyce, T. Peoples, B.A. Perkins, J.B. Welsh, Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes, *N. Engl. J. Med.* 363 (2010) 311–320.
- D.R. Owens, B. Zinman, G. Bolli, Alternative routes of insulin delivery, *Diabet. Med.* 20 (2003) 886–898.
- A. des Rieux, V. Fievez, M. Garinot, Y.-J. Schneider, V. Préat, Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach, *J. Control. Release* 116 (2006) 1–27.
- A.J. Almeida, E. Souto, Solid lipid nanoparticles as a drug delivery system for peptides and proteins, *Adv. Drug Deliv. Rev.* 59 (2007) 478–490.
- J.S. Patton, P.R. Byron, Inhaling medicines: delivering drugs to the body through the lungs, *Nat. Rev. Drug Discov.* 6 (2007) 67.
- X. Chen, L. Wang, H.J. Yu, C.J. Li, J.Y. Feng, F. Haq, A. Khan, R.U. Khan, Preparation, properties and challenges of the microneedles-based insulin delivery system, *J. Control. Release* 288 (2018) 173–188.
- F. Zhao, D. Wu, D. Yao, R. Guo, W. Wang, A. Dong, D. Kong, J. Zhang, An injectable particle-hydrogel hybrid system for glucose-regulatory insulin delivery, *Acta Biomater.* 64 (2017) 334–345.
- Q. Wu, L. Wang, H. Yu, J. Wang, Z. Chen, Organization of glucose-responsive systems and their properties, *Chem. Rev.* 111 (2011) 7855–7875.
- Y. Qiu, K. Park, Environment-sensitive hydrogels for drug delivery, *Adv. Drug Deliv. Rev.* 53 (2001) 321–339.
- J. Kost, R. Langer, Responsive polymeric delivery systems, *Adv. Drug Deliv. Rev.* 64 (2012) 327–341.
- K.G.M.M. Alberti, P.f. Zimmet, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation, *Diabet. Med.* 15 (1998) 539–553.
- A. Faria, S.J. Persaud, Cardiac oxidative stress in diabetes: mechanisms and therapeutic potential, *Pharmacol. Ther.* 172 (2017) 50–62.
- N. Napoli, M. Chandran, D.D. Pierroz, B. Abrahamsen, A.V. Schwartz, S.L. Ferrari, Mechanisms of diabetes mellitus-induced bone fragility, *Nat. Rev. Endocrinol.* 13 (2017) 208.
- N.M. Cheung, P. Wong, TY, Diabetic retinopathy, *The Lancet* 376 (2010) 124–136.
- D.S. van, J.W. Beulens, Y.T. Van der Schouw, D.E. Grobbee, B. Nealb, The global burden of diabetes and its complications: an emerging pandemic, *Eur. J. Cardiovasc. Prev. Rehabil* 17 (2010) s3–s8.
- S. Wild, G. Roglic, A. Green, R. Sicree, H. King, Global prevalence of diabetes: estimates for the year 2000 and projections for 2030, *Diabetes Care* 27 (2004) 1047–1053.
- A. Chaudhury, C. Duvoor, R. Dendi, V. Sena, S. Kraleti, A. Chada, R. Ravilla, A. Marco, N.S. Shekhawat, M.T. Montales, Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management, *Front. Endocrinol.* 8 (2017) 6.
- C. Hampf, V. Borders-Hemphill, D.G. Moeny, D.K. Wysowski, Use of antidiabetic drugs in the US, 2003–2012, *Diabetes Care* 37 (2014) 1367–1374.
- D. Control, C.T.R. Group, The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus, *N. Engl. J. Med.* 329 (1993) 977–986.
- E. Ferrannini, R.A. DeFronzo, Impact of glucose-lowering drugs on cardiovascular disease in type 2 diabetes, *Eur. Heart J.* 36 (2015) 2288–2296.
- S.E. Kahn, M.E. Cooper, S. Del Prato, Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future, *Lancet* 383 (2014) 1068–1083.
- R. Mo, T. Jiang, J. Di, W. Tai, Z. Gu, Emerging micro- and nanotechnology based synthetic approaches for insulin delivery, *Chem. Soc. Rev.* 43 (2014) 3595–3629.
- M. Goldberg, I. Gomez-Orellana, Challenges for the oral delivery of macromolecules, *Nat. Rev. Drug Discov.* 2 (2003) 289.
- S. Mitragotri, P.A. Burke, R. Langer, Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies, *Nat. Rev. Drug Discov.* 13 (2014) 655.
- M. Morishita, N.A. Peppas, Is the oral route possible for peptide and protein drug delivery? *Drug Discov. Today* 11 (2006) 905–910.
- E.-S. Khafagy, M. Morishita, Y. Onuki, K. Takayama, Current challenges in non-invasive insulin delivery systems: a comparative review, *Adv. Drug Deliv. Rev.* 59 (2007) 1521–1546.
- D. Porte, A. Pupo, Insulin responses to glucose: evidence for a two pool system in man, *J. Clin. Invest.* 48 (1969) 2309–2319.
- F. Ismail-Beigi, Pathogenesis and glycemic management of type 2 diabetes mellitus: a physiological approach, *Arch. Iran. Med.* 15 (2012) 239–246.
- L. Poretsky, Principles of Diabetes Mellitus, Springer, New York, 2010.
- X. Guo, W. Wang, Challenges and recent advances in the subcutaneous delivery of insulin, *Expert Opin. Drug Delivery* 14 (2017) 727–734.
- X. Jin, D.D. Zhu, B.Z. Chen, M. Ashfaq, X.D. Guo, Insulin delivery systems combined with microneedle technology, *Adv. Drug Deliv. Rev.* 127 (2018) 119–137.
- Y.-J. Lin, F.-L. Mi, P.-Y. Lin, Y.-B. Miao, T. Huang, K.-H. Chen, C.-T. Chen, Y. Chang, H.-W. Sung, Strategies for improving diabetic therapy via alternative administration routes that involve stimuli-responsive insulin-delivering systems, *Adv. Drug Deliv. Rev.* 139 (2019) 71–82.
- J.-Z. Wu, G.R. Williams, H.-Y. Li, D.-X. Wang, S.-D. Li, L.-M. Zhu, Insulin-loaded PLGA microspheres for glucose-responsive release, *Drug Deliv.* 24 (2017) 1513–1525.
- L. Hou, Y. Zheng, Y. Wang, Y. Hu, J. Shi, Q. Liu, H. Zhang, Z. Zhang, Self-regulated carboxyphenylboronic acid-modified mesoporous silica nanoparticles with “touch switch” releasing property for insulin delivery, *ACS Appl. Mater. Interfaces* 10 (2018) 21927–21938.
- L. Zhao, L. Niu, H. Liang, H. Tan, C. Liu, F. Zhu, pH and glucose dual-responsive injectable hydrogels with insulin and fibroblasts as bioactive dressings for diabetic wound healing, *ACS Appl. Mater. Interfaces* 9 (2017) 37563–37574.
- M.R. Prausnitz, Microneedles for transdermal drug delivery, *Adv. Drug Deliv. Rev.* 56 (2004) 581–587.
- Y. Yu, Y. Zhang, Y. Ye, R. DiSanto, W. Sun, D. Ranson, F.S. Ligler, J.B. Buse, Z. Gu, Microneedle-array patches loaded with hypoxia-sensitive vesicles provide fast glucose-responsive insulin delivery, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 8260–8265.
- C.Y. Wong, H. Al-Salami, C.R. Dass, Recent advancements in oral administration of insulin-loaded liposomal drug delivery systems for diabetes mellitus, *Int. J. Pharm.* 549 (2018) 201–217.
- C.Y. Wong, J. Martinez, C.R. Dass, Oral delivery of insulin for treatment of diabetes: status quo, challenges and opportunities, *J. Pharm. Pharmacol.* 68 (2016) 1093–1108.
- M.-C. Chen, K. Sonaje, K.-J. Chen, H.-W. Sung, A review of the prospects for polymeric nanoparticle platforms in oral insulin delivery, *Biomaterials* 32 (2011) 9826–9838.
- W. Shan, X. Zhu, M. Liu, L. Li, J. Zhong, W. Sun, Z. Zhang, Y. Huang, Overcoming the diffusion barrier of mucus and absorption barrier of epithelium by self-assembled nanoparticles for oral delivery of insulin, *ACS Nano* 9 (2015) 2345–2356.
- W. Fan, D. Xia, Q. Zhu, X. Li, S. He, C. Zhu, S. Guo, L. Hovgaard, M. Yang, Y. Gan, Functional nanoparticles exploit the bile acid pathway to overcome multiple barriers of the intestinal epithelium for oral insulin delivery, *Biomaterials* 151 (2018) 13–23.
- F. Andrade, P. Fonte, A. Costa, C.C. Reis, R. Nunes, A. Almeida, D. Ferreira, M. Oliva, B. Sarmento, Pharmacological and toxicological assessment of innovative self-assembled polymeric micelles as powders for insulin pulmonary delivery, *Nanomedicine* 11 (2016) 2305–2317.
- E. Pringels, C. Callens, C. Vervaet, F. Dumont, G. Slegers, P. Foreman, J.P. Remon, Influence of deposition and spray pattern of nasal powders on insulin

- bioavailability, *Int. J. Pharm.* 310 (2006) 1–7.
- [51] R. Soane, M. Hinchcliffe, S. Davis, L. Illum, Clearance characteristics of chitosan based formulations in the sheep nasal cavity, *Int. J. Pharm.* 217 (2001) 183–191.
- [52] Y. Zhang, X.-G. Jiang, J. Yao, Nasal absorption enhancement of insulin by sodium deoxycholate in combination with cyclodextrins, *Acta Pharmacol. Sin.* 22 (2001) 1051–1056.
- [53] J.H. Holtz, S.A. Asher, Polymerized colloidal crystal hydrogel films as intelligent chemical sensing materials, *Nature* 389 (1997) 829.
- [54] D. Roy, J.N. Cambre, B.S. Sumerlin, Future perspectives and recent advances in stimuli-responsive materials, *Prog. Polym. Sci.* 35 (2010) 278–301.
- [55] Y. Zhao, B.G. Trewyn, I.I. Slowing, V.S.-Y. Lin, Mesoporous silica nanoparticle-based double drug delivery system for glucose-responsive controlled release of insulin and cyclic AMP, *J. Am. Chem. Soc.* 131 (2009) 8398–8400.
- [56] V. Yesilyurt, M.J. Webber, E.A. Appel, C. Godwin, R. Langer, D.G. Anderson, Injectable self-healing glucose-responsive hydrogels with pH-regulated mechanical properties, *Adv. Mater.* 28 (2016) 86–91.
- [57] K. Besteman, J.-O. Lee, F.G. Wiertz, H.A. Heering, C. Dekker, Enzyme-coated carbon nanotubes as single-molecule biosensors, *Nano Lett.* 3 (2003) 727–730.
- [58] V. Ravaine, C. Ancla, B. Catargi, Chemically controlled closed-loop insulin delivery, *J. Control. Release* 132 (2008) 2–11.
- [59] G. Springsteen, B. Wang, A detailed examination of boronic acid-diol complexation, *Tetrahedron* 58 (2002) 5291–5300.
- [60] Y. Zhu, W. Tong, C. Gao, Molecular-engineered polymeric microcapsules assembled from Concanavalin A and glycogen with specific responses to carbohydrates, *Soft Matter* 7 (2011) 5805–5815.
- [61] J. Wang, Z. Wang, J. Yu, A.R. Kahkoska, J.B. Buse, Z. Gu, Glucose-responsive insulin and delivery systems: innovation and translation, *Adv. Mater.* 1902004 (2019).
- [62] Q. Huang, L. Wang, H. Yu, K. Ur-Rahman, Advances in phenylboronic acid-based closed-loop smart drug delivery system for diabetic therapy, *J. Control. Release* 305 (2019) 50–64.
- [63] W.L. Brooks, C.C. Deng, B.S. Sumerlin, Structure-reactivity relationships in boronic acid-diol complexation, *ACS Omega* 3 (2018) 17863–17870.
- [64] A. Matsumoto, S. Ikeda, A. Harada, K. Kataoka, Glucose-responsive polymer bearing a novel phenylborate derivative as a glucose-sensing moiety operating at physiological pH conditions, *Biomacromolecules* 4 (2003) 1410–1416.
- [65] A. Matsumoto, T. Ishii, J. Nishida, H. Matsumoto, K. Kataoka, Y. Miyahara, A synthetic approach toward a self-regulated insulin delivery system, *Angew. Chem. Int. Ed.* 51 (2012) 2124–2128.
- [66] D.H. McDANIEL, H.C. Brown, An extended table of hammett substituent constants based on the ionization of substituted benzoic acids, *J. Organomet. Chem.* 23 (1958) 420–427.
- [67] X. Li, J. Pennington, J.F. Stobaugh, C. Schöneich, Synthesis of sulfonamide- and sulfonyl-phenylboronic acid-modified silica phases for boronate affinity chromatography at physiological pH, *Anal. Biochem.* 372 (2008) 227–236.
- [68] Y. Liu, L. Ren, Z. Liu, A unique boronic acid functionalized monolithic capillary for specific capture, separation and immobilization of *cis*-diol biomolecules, *Chem. Commun.* 47 (2011) 5067–5069.
- [69] J. Yan, G. Springsteen, S. Deeter, B. Wang, The relationship among pK_a , pH, and binding constants in the interactions between boronic acids and diols—it is not as simple as it appears, *Tetrahedron* 60 (2004) 11205–11209.
- [70] R. Singhal, B. Ramamurthy, N. Govindraj, Y. Sarwar, New ligands for boronate affinity chromatography synthesis and properties, *J. Chromatogr.* 543 (1991) 17–38.
- [71] C. Hansch, A. Leo, R. Taft, A survey of Hammett substituent constants and resonance and field parameters, *Chem. Rev.* 91 (1991) 165–195.
- [72] H. Li, Y. Liu, J. Liu, Z. Liu, A Wulff-type boronate for boronate affinity capture of *cis*-diol compounds at medium acidic pH condition, *Chem. Commun.* 47 (2011) 8169–8171.
- [73] G. Wulff, Selective binding to polymers via covalent bonds. The construction of chiral cavities as specific receptor sites, *Pure Appl. Chem.* 54 (1982) 2093–2102.
- [74] L. Zhu, S.H. Shabbir, M. Gray, V.M. Lynch, S. Corey, E.V. Anslyn, A structural investigation of the N-B interaction in an *o*-(*N*, *N*-dialkylaminomethyl) arylboronate system, *J. Am. Chem. Soc.* 128 (2006) 1222–1232.
- [75] Z.H. Farooqi, W. Wu, S. Zhou, M. Siddiq, Engineering of phenylboronic acid based glucose-sensitive microgels with 4-vinylpyridine for working at physiological pH and temperature, *Macromol. Chem. Phys.* 212 (2011) 1510–1514.
- [76] L. Liang, Z. Liu, A self-assembled molecular team of boronic acids at the gold surface for specific capture of *cis*-diol biomolecules at neutral pH, *Chem. Commun.* 47 (2011) 2255–2257.
- [77] L. Ren, Z. Liu, Y. Liu, P. Dou, H.Y. Chen, Ring-opening polymerization with synergistic co-monomers: access to a boronate-functionalized polymeric monolith for the specific capture of *cis*-diol-containing biomolecules under neutral conditions, *Angew. Chem.* 121 (2009) 6832–6835.
- [78] C.C. Deng, W.L. Brooks, K.A. Abboud, B.S. Sumerlin, Boronic acid-based hydrogels undergo self-healing at neutral and acidic pH, *ACS Macro Lett.* 4 (2015) 220–224.
- [79] M. Dowlut, D.G. Hall, An improved class of sugar-binding boronic acids, soluble and capable of complexing glycosides in neutral water, *J. Am. Chem. Soc.* 128 (2006) 4226–4227.
- [80] Y. Chen, D. Diaz-Dussan, D. Wu, W. Wang, Y.-Y. Peng, A.B. Asha, D.G. Hall, K. Ishihara, R. Narain, Bioinspired self-healing hydrogel based on benzoxaborole-catechol dynamic covalent chemistry for 3D cell encapsulation, *ACS Macro Lett.* 7 (2018) 904–908.
- [81] Y. Dong, W. Wang, O. Veiseh, E.A. Appel, K. Xue, M.J. Webber, B.C. Tang, X.-W. Yang, G.C. Weir, R. Langer, Injectable and glucose-responsive hydrogels based on boronic acid–glucose complexation, *Langmuir* 32 (2016) 8743–8747.
- [82] W. Sun, H. Jiang, X. Wu, Z. Xu, C. Yao, J. Wang, M. Qin, Q. Jiang, W. Wang, D. Shi, Strong dual-crosslinked hydrogels for ultrasound-triggered drug delivery, *Nano Res.* 12 (2019) 115–119.
- [83] D. Keilin, E. Hartree, Properties of glucose oxidase (notatin): addendum. Sedimentation and diffusion of glucose oxidase (notatin), *Biochem. J.* 42 (1948) 221.
- [84] C.M. Wong, K.H. Wong, X.D. Chen, Glucose oxidase: natural occurrence, function, properties and industrial applications, *Appl. Microbiol. Biotechnol.* 78 (2008) 927–938.
- [85] D. Goodsell, Glucose oxidase, Molecule of the month: PCSB Protein Data Bank, 2006.
- [86] H. Kalisz, J. Hendle, R. Schmid, Structural and biochemical properties of glycosylated and deglycosylated glucose oxidase from *Penicillium amagasakiense*, *Appl. Microbiol. Biotechnol.* 47 (1997) 502–507.
- [87] S. Nakamura, S. FUJIKI, Comparative studies on the glucose oxidases of *Aspergillus niger* and *Penicillium amagasakiense*, *J. Biochem.* 63 (1968) 51–58.
- [88] B.E. Swoboda, V. Massey, Purification and properties of the glucose oxidase from *Aspergillus niger*, *J. Biol. Chem.* 240 (1965) 2209.
- [89] H. Tsuge, O. Natsuaki, K. Ohashi, Purification, properties, and molecular features of glucose oxidase from *Aspergillus niger*, *J. Biochem.* 78 (1975) 835–843.
- [90] V. Leskovic, S. Trivić, G. Wohlfahrt, J. Kandrač, D. Peričin, Glucose oxidase from *Aspergillus niger*: the mechanism of action with molecular oxygen, quinones, and one-electron acceptors, *Int. J. Biochem. Cell Biol.* 37 (2005) 731–750.
- [91] O. Syshchik, V.A. Skryshevsky, O.O. Soldatkin, A.P. Soldatkin, Enzyme biosensor systems based on porous silicon photoluminescence for detection of glucose, urea and heavy metals, *Biosens. Bioelectron.* 66 (2015) 89–94.
- [92] S. Nakamura, Y. Ogura, Mode of inhibition of glucose oxidase by metal ions, *J. Biochem.* 64 (1968) 439–447.
- [93] E.C. Toren, F.J. Burger, Trace determination of metal ion inhibitors of the glucose-glucose oxidase system, *Microchim. Acta* 56 (1968) 538–545.
- [94] Q.H. Gibson, B.E. Swoboda, V. Massey, Kinetics and mechanism of action of glucose oxidase, *J. Biol. Chem.* 239 (1964) 3927–3934.
- [95] S. Nakamura, Y. Ogura, Action mechanism of glucose oxidase of *Aspergillus niger*, *J. Biochem.* 63 (1968) 308–316.
- [96] J. Bao, K. Furumoto, K. Fukunaga, K. Nakao, A kinetic study on air oxidation of glucose catalyzed by immobilized glucose oxidase for production of calcium gluconate, *Biochem. Eng. J.* 8 (2001) 91–102.
- [97] S.B. Bankar, M.V. Bule, R.S. Singhal, L. Ananthanarayan, Glucose oxidase—an overview, *Biotechnol. Adv.* 27 (2009) 489–501.
- [98] X. Wu, J. Ge, C. Yang, M. Hou, Z. Liu, Facile synthesis of multiple enzyme-containing metal-organic frameworks in a biomolecule-friendly environment, *Chem. Commun.* 51 (2015) 13408–13411.
- [99] J. Wang, Y. Ye, J. Yu, A.R. Kahkoska, X. Zhang, C. Wang, W. Sun, R.D. Corder, Z. Chen, S.A. Khan, Core-shell microneedle gel for self-regulated insulin delivery, *ACS Nano* 12 (2018) 2466–2473.
- [100] Q. Li, B. Xia, M. Branham, W. Ha, H. Wu, S.-L. Peng, L.-S. Ding, B.-J. Li, S. Zhang, Self-assembly of carboxymethyl konjac glucomannan-g-poly (ethylene glycol) and (α -cyclodextrin) to biocompatible hollow nanospheres for glucose oxidase encapsulation, *Carbohydr. Polym.* 86 (2011) 120–126.
- [101] A. Napoli, M.J. Boerakker, N. Tirelli, R.J. Nolte, N.A. Sommerdijk, J.A. Hubbell, Glucose-oxidase based self-destructing polymeric vesicles, *Langmuir* 20 (2004) 3487–3491.
- [102] W. Qi, X. Yan, J. Fei, A. Wang, Y. Cui, J. Li, Triggered release of insulin from glucose-sensitive enzyme multilayer shells, *Biomaterials* 30 (2009) 2799–2806.
- [103] R. Qu, L. Shen, A. Qu, R. Wang, Y. An, L. Shi, Artificial peroxidase/oxidase multiple enzyme system based on supramolecular hydrogel and its application as a biocatalyst for cascade reactions, *ACS Appl. Mater. Interfaces* 7 (2015) 16694–16705.
- [104] W. Wu, S. Zhou, Responsive materials for self-regulated insulin delivery, *Macromol. Biosci.* 13 (2013) 1464–1477.
- [105] D.B. Jones, C.O. Johns, Some proteins from the jack bean, *Canavalia ensiformis*, *J. Biol. Chem.* 28 (1916) 67–75.
- [106] W. Li, J. Yu, H. Xu, J. Bao, Concanavalin A: a potential anti-neoplastic agent targeting apoptosis, autophagy and anti-angiogenesis for cancer therapeutics, *Biochem. Biophys. Res. Commun.* 414 (2011) 282–286.
- [107] G.M. Edelman, B.A. Cunningham, G.N. Reeke, J.W. Becker, M.J. Waxdal, J.L. Wang, The covalent and three-dimensional structure of concanavalin A, *Proc. Natl. Acad. Sci. U. S. A.* 69 (1972) 2580–2584.
- [108] J. Huang, L. Zhang, R.-P. Liang, J.-D. Qiu, “On-off” switchable electrochemical affinity nanobiosensor based on graphene oxide for ultrasensitive glucose sensing, *Biosens. Bioelectron.* 41 (2013) 430–435.
- [109] Y. Song, H. Liu, H. Tan, F. Xu, J. Jia, L. Zhang, Z. Li, L. Wang, pH-switchable electrochemical sensing platform based on chitosan-reduced graphene oxide/concanavalin A layer for assay of glucose and urea, *Anal. Chem.* 86 (2014) 1980–1987.
- [110] B. Wang, J.-i. Anzai, Recent progress in lectin-based biosensors, *Materials* 8 (2015) 8590–8607.
- [111] L.D. Sappia, E. Piccinini, W. Marmisollé, N. Santilli, E. Maza, S. Moya, F. Battaglini, R.E. Madrid, O. Azzaroni, Integration of biorecognition elements on PEDOT platforms through supramolecular interactions, *Adv. Mater. Interfaces* 4 (2017) 1700502.
- [112] S. Wu, X. Huang, X. Du, Glucose- and pH-responsive controlled release of cargo from protein-gated carbohydrate-functionalized mesoporous silica nanocontainers, *Angew. Chem.* 125 (2013) 5690–5694.
- [113] K. Sun, S.A. Blich, H.-l. Nie, J. Quan, L.-m. Zhu, Lectin recognizing thermo-responsive double hydrophilic copolymer micelles by RAFT polymerization,

- RSC Adv. 4 (2014) 34912–34921.
- [114] Y. Wang, Z. Chen, Y. Liu, J. Li, A functional glycoprotein competitive recognition and signal amplification strategy for carbohydrate-protein interaction profiling and cell surface carbohydrate expression evaluation, *Nanoscale* 5 (2013) 7349–7355.
- [115] M. Martínez-Carmona, D. Lozano, M. Colilla, M. Vallet-Regí, Lectin-conjugated pH-responsive mesoporous silica nanoparticles for targeted bone cancer treatment, *Acta Biomater.* 65 (2018) 393–404.
- [116] B. Li, A. Yu, G. Lai, Self-assembly of phenoxyl-dextran on electrochemically reduced graphene oxide for nonenzymatic biosensing of glucose, *Carbon* 127 (2018) 202–208.
- [117] M.A. VandenBerg, M.J. Webber, Biologically inspired and chemically derived methods for glucose-responsive insulin therapy, *Adv. Healthcare Mater.* 1801466 (2019).
- [118] T. Miyata, A. Jikihara, K. Nakamae, A.S. Hoffman, Preparation of reversibly glucose-responsive hydrogels by covalent immobilization of lectin in polymer networks having pendant glucose, *J. Biomater. Sci. Polym. Ed.* 15 (2004) 1085–1098.
- [119] D.H.-C. Chou, M.J. Webber, B.C. Tang, A.B. Lin, L.S. Thapa, D. Deng, J.V. Truong, A.B. Cortinas, R. Langer, D.G. Anderson, Glucose-responsive insulin activity by covalent modification with aliphatic phenylboronic acid conjugates, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 2401–2406.
- [120] X. Xu, H. Shang, T. Zhang, P. Shu, Y. Liu, J. Xie, D. Zhang, H. Tan, J. Li, A stimuli-responsive insulin delivery system based on reversible phenylboronate modified cyclodextrin with glucose triggered host-guest interaction, *Int. J. Pharm.* 548 (2018) 649–658.
- [121] L. Zhao, C. Xiao, J. Ding, X. Zhuang, G. Gai, L. Wang, X. Chen, Competitive binding-accelerated insulin release from a polypeptide nanogel for potential therapy of diabetes, *Polym. Chem.* 6 (2015) 3807–3815.
- [122] M. Oroval, P. Díez, E. Aznar, C. Coll, M.D. Marcos, F. Sancenón, R. Villalonga, R. Martínez-Mañez, Self-regulated glucose-sensitive neoglycoenzyme-capped mesoporous silica nanoparticles for insulin delivery, *Chem. Eur. J.* 23 (2017) 1353–1360.
- [123] Y. Fu, W. Liu, L.Y. Wang, B.Y. Zhu, M.K. Qu, L.Q. Yang, X. Sun, T. Gong, Z.R. Zhang, Q. Lin, Erythrocyte-membrane-camouflaged nanoplatfor for intravenous glucose-responsive insulin delivery, *Adv. Funct. Mater.* 28 (2018) 1802250.
- [124] C. Li, F. Huang, Y. Liu, J. Lv, G. Wu, Y. Liu, R. Ma, Y. An, L. Shi, Nitrotriacetic acid-functionalized glucose-responsive complex micelles for the efficient encapsulation and self-regulated release of insulin, *Langmuir* 34 (2018) 12116–12125.
- [125] W. Tai, R. Mo, J. Di, V. Subramanian, X. Gu, J.B. Buse, Z. Gu, Bio-inspired synthetic nanovesicles for glucose-responsive release of insulin, *Biomacromolecules* 15 (2014) 3495–3502.
- [126] Y. Duan, F. Ye, Y. Huang, Y. Qin, C. He, S. Zhao, One-pot synthesis of a metal-organic framework-based drug carrier for intelligent glucose-responsive insulin delivery, *Chem. Commun.* 54 (2018) 5377–5380.
- [127] J. Di, J. Yu, Y. Ye, D. Ranson, A. Jindal, Z. Gu, Engineering synthetic insulin-secreting cells using hyaluronic acid microgels integrated with glucose-responsive nanoparticles, *Cell. Mol. Bioeng.* 8 (2015) 445–454.
- [128] M. Zhang, C.-C. Song, F.-S. Du, Z.-C. Li, Supersensitive oxidation-responsive biodegradable PEG hydrogels for glucose-triggered insulin delivery, *ACS Appl. Mater. Interfaces* 9 (2017) 25905–25914.
- [129] S. Chen, H. Matsumoto, Y. Moro-oka, M. Tanaka, Y. Miyahara, T. Suganami, A. Matsumoto, Microneedle-array patch fabricated with enzyme-free polymeric components capable of on-demand insulin delivery, *Adv. Funct. Mater.* 29 (2019) 1807369.
- [130] A. Matsumoto, M. Tanaka, H. Matsumoto, K. Ochi, Y. Moro-oka, H. Kuwata, H. Yamada, I. Shirakawa, T. Miyazawa, H. Ishii, Synthetic “smart gel” provides glucose-responsive insulin delivery in diabetic mice, *Sci. Adv.* 3 (2017) eaaq0723.
- [131] T. Seki, K. Abe, Y. Egawa, R. Miki, K. Juni, T. Seki, A pseudopolyrotaxane for glucose-responsive insulin release: the effect of binding ability and spatial arrangement of phenylboronic acid group, *Mol. Pharm.* 13 (2016) 3807–3815.
- [132] R. Chang, M. Li, S. Ge, J. Yang, Q. Sun, L. Xiong, Glucose-responsive biopolymer nanoparticles prepared by co-assembly of concanavalin A and amylopectin for insulin delivery, *Ind. Crop. Prod.* 112 (2018) 98–104.
- [133] B. Gu, D.J. Burgess, Prediction of dexamethasone release from PLGA microspheres prepared with polymer blends using a design of experiment approach, *Int. J. Pharm.* 495 (2015) 393–403.
- [134] Y.-Y. Yang, T.-S. Chung, X.-L. Bai, W.-K. Chan, Effect of preparation conditions on morphology and release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method, *Chem. Eng. Sci.* 55 (2000) 2223–2236.
- [135] H.K. Kim, T.G. Park, Microencapsulation of human growth hormone within biodegradable polyester microspheres: protein aggregation stability and incomplete release mechanism, *Biotechnol. Bioeng.* 65 (1999) 659–667.
- [136] M. Bai, J. He, L. Kang, J. Nie, R. Yin, Regulated basal and bolus insulin release from glucose-responsive core-shell microspheres based on concanavalin A-sugar affinity, *Int. J. Biol. Macromol.* 113 (2018) 889–899.
- [137] C. Xu, C. Lei, L. Huang, J. Zhang, H. Song, M. Yu, Y. Wu, C. Chen, C. Yu, Glucose-responsive nanosystem mimicking the physiological insulin secretion via an enzyme-polymer layer-by-layer coating strategy, *Chem. Mater.* 29 (2017) 7725–7732.
- [138] P. Díez, B.E.-F. de Ávila, D.E. Ramírez-Herrera, R. Villalonga, J. Wang, Biomedical nanomotors: efficient glucose-mediated insulin release, *Nanoscale* 9 (2017) 14307–14311.
- [139] Z.W. Lim, Y. Ping, A. Miserez, Glucose-responsive peptide coacervates with high encapsulation efficiency for controlled release of insulin, *Bioconjug. Chem.* 29 (2018) 2176–2180.
- [140] R. Yin, K. Wang, S. Du, L. Chen, J. Nie, W. Zhang, Design of genipin-crosslinked microgels from concanavalin A and glucosyloxyethyl acrylated chitosan for glucose-responsive insulin delivery, *Carbohydr. Polym.* 103 (2014) 369–376.
- [141] C. Li, X. Liu, Y. Liu, F. Huang, G. Wu, Y. Liu, Z. Zhang, Y. Ding, J. Lv, R. Ma, Glucose and H₂O₂ dual-sensitive nanogels for enhanced glucose-responsive insulin delivery, *Nanoscale* 11 (2019) 9163–9175.
- [142] H. Wang, J. Yi, Y. Yu, S. Zhou, NIR upconversion fluorescence glucose sensing and glucose-responsive insulin release of carbon dot-immobilized hybrid microgels at physiological pH, *Nanoscale* 9 (2017) 509–516.
- [143] T. Ye, S. Yan, Y. Hu, L. Ding, W. Wu, Synthesis and volume phase transition of concanavalin A-based glucose-responsive nanogels, *Polym. Chem.* 5 (2014) 186–194.
- [144] D. Lee, K. Choe, Y. Jeong, J. Yoo, S.M. Lee, J.-H. Park, P. Kim, Y.-C. Kim, Establishment of a controlled insulin delivery system using a glucose-responsive double-layered nanogel, *RSC Adv.* 5 (2015) 14482–14491.
- [145] C. Wang, Y. Ye, W. Sun, J. Yu, J. Wang, D.S. Lawrence, J.B. Buse, Z. Gu, Red blood cells for glucose-responsive insulin delivery, *Adv. Mater.* 29 (2017) 1606617.
- [146] N. Wen, C. Gao, S. Lü, X. Xu, X. Bai, C. Wu, P. Ning, S. Zhang, M. Liu, Novel amphiphilic glucose-responsive modified starch micelles for insulin delivery, *RSC Adv.* 7 (2017) 45978–45986.
- [147] D. Shi, M. Ran, L. Zhang, H. Huang, X. Li, M. Chen, M. Akashi, Fabrication of biobased polyelectrolyte capsules and their application for glucose-triggered insulin delivery, *ACS Appl. Mater. Interfaces* 8 (2016) 13688–13697.
- [148] W. Yuan, T. Shen, J. Wang, H. Zou, Formation-dissociation of glucose, pH and redox triply responsive micelles and controlled release of insulin, *Polym. Chem.* 5 (2014) 3968–3971.
- [149] X. Li, H. Shang, W. Wu, S. Li, Z. Lin, J. Duan, L. Xu, J. Li, Glucose-responsive micelles for controlled insulin release based on transformation from amphiphilic to double hydrophilic, *J. Nanosci. Nanotechnol.* 16 (2016) 5457–5463.
- [150] X. Du, G. Jiang, L. Li, Y. Liu, H. Chen, Q. Huang, Photo-induced synthesis glucose-responsive carriers for controlled release of insulin *in vitro*, *Colloid Polym. Sci.* 293 (2015) 2129–2135.
- [151] W. Yuan, L. Li, H. Zou, Thermo-and glucose-responsive micelles self-assembled from phenylborate ester-containing brush block copolymer for controlled release of insulin at physiological pH, *RSC Adv.* 5 (2015) 80264–80268.
- [152] N. Wen, S. Lü, C. Gao, X. Xu, X. Bai, C. Wu, P. Ning, M. Liu, Glucose-responsive zwitterionic dialdehyde starch-based micelles with potential anti-phagocytic behavior for insulin delivery, *Chem. Eng. J.* 335 (2018) 52–62.
- [153] L. Gao, T. Wang, K. Jia, X. Wu, C. Yao, W. Shao, D. Zhang, X.Y. Hu, L. Wang, Glucose-responsive supramolecular vesicles based on water-soluble pillar [5] arene and pyridylboronic acid derivatives for controlled insulin delivery, *Chem. Eur. J.* 23 (2017) 6605–6614.
- [154] Z. Chai, L. Ma, Y. Wang, X. Ren, Phenylboronic acid as a glucose-responsive trigger to tune the insulin release of glycopolymer nanoparticles, *J. Biomater. Sci. Polym. Ed.* 27 (2016) 599–610.
- [155] D. Xia, H. He, Y. Wang, K. Wang, H. Zuo, H. Gu, P. Xu, Y. Hu, Ultrafast glucose-responsive, high loading capacity erythrocyte to self-regulate the release of insulin, *Acta Biomater.* 69 (2018) 301–312.
- [156] L.R. Volpatti, M.A. Matranga, A.B. Cortinas, D. Delcassian, K.B. Daniel, R. Langer, D.G. Anderson, Glucose-responsive nanoparticles for rapid and extended self-regulated insulin delivery, *ACS Nano* 14 (2019) 488–497.
- [157] M.C. Koetting, J.T. Peters, S.D. Steichen, N.A. Peppas, Stimulus-responsive hydrogels: theory, modern advances, and applications, *Mater. Sci. Eng. R* 93 (2015) 1–49.
- [158] Z. Gu, T.T. Dang, M. Ma, B.C. Tang, H. Cheng, S. Jiang, Y. Dong, Y. Zhang, D.G. Anderson, Glucose-responsive microgels integrated with enzyme nanoparticles for closed-loop insulin delivery, *ACS Nano* 7 (2013) 6758–6766.
- [159] Y. Ye, J. Yu, D. Wen, A.R. Kahkoska, Z. Gu, Polymeric microneedles for transdermal protein delivery, *Adv. Drug Deliv. Rev.* 127 (2018) 106–118.
- [160] B. Kim, N.A. Peppas, *In vitro* release behavior and stability of insulin in complexation hydrogels as oral drug delivery carriers, *Int. J. Pharm.* 266 (2003) 29–37.
- [161] J. Lee, J.H. Ko, K.M. Mansfield, P.C. Nauka, E. Bat, H.D. Maynard, Glucose-responsive trehalose hydrogel for insulin stabilization and delivery, *Macromol. Biosci.* 18 (2018) 1700372.
- [162] L. Li, G. Jiang, W. Yu, D. Liu, H. Chen, Y. Liu, Q. Huang, Z. Tong, J. Yao, X. Kong, A composite hydrogel system containing glucose-responsive nanocarriers for oral delivery of insulin, *Mater. Sci. Eng. C* 69 (2016) 37–45.
- [163] X. Li, M. Fu, J. Wu, C. Zhang, X. Deng, A. Dhinakar, W. Huang, H. Qian, L. Ge, pH-sensitive peptide hydrogel for glucose-responsive insulin delivery, *Acta Biomater.* 51 (2017) 294–303.
- [164] B. Xu, Q. Cao, Y. Zhang, W. Yu, J. Zhu, D. Liu, G. Jiang, Microneedles integrated with ZnO quantum-dot-capped mesoporous bioactive glasses for glucose-mediated insulin delivery, *ACS Biomater. Sci. Eng.* 4 (2018) 2473–2483.
- [165] Y. Ye, J. Yu, C. Wang, N.Y. Nguyen, G.M. Walker, J.B. Buse, Z. Gu, Microneedles integrated with pancreatic cells and synthetic glucose-signal amplifiers for smart insulin delivery, *Adv. Mater.* 28 (2016) 3115–3121.
- [166] X. Hu, J. Yu, C. Qian, Y. Lu, A.R. Kahkoska, Z. Xie, X. Jing, J.B. Buse, Z. Gu, H₂O₂-responsive vesicles integrated with transcutaneous patches for glucose-mediated insulin delivery, *ACS Nano* 11 (2017) 613–620.
- [167] B. Xu, G. Jiang, W. Yu, D. Liu, Y. Zhang, J. Zhou, S. Sun, Y. Liu, H₂O₂-responsive mesoporous silica nanoparticles integrated with microneedle patches for the glucose-monitored transdermal delivery of insulin, *J. Mater. Chem. B* 5 (2017) 8200–8208.

- [168] Z. Tong, J. Zhou, J. Zhong, Q. Tang, Z. Lei, H. Luo, P. Ma, X. Liu, Glucose- and H₂O₂-responsive polymeric vesicles integrated with microneedle patches for glucose-sensitive transcutaneous delivery of insulin in diabetic rats, *ACS Appl. Mater. Interfaces* 10 (2018) 20014–20024.
- [169] J. Yu, C. Qian, Y. Zhang, Z. Cui, Y. Zhu, Q. Shen, F.S. Ligler, J.B. Buse, Z. Gu, Hypoxia and H₂O₂ dual-sensitive vesicles for enhanced glucose-responsive insulin delivery, *Nano Lett.* 17 (2017) 733–739.
- [170] Y. Zhang, J. Wang, J. Yu, D. Wen, A.R. Kahkoska, Y. Lu, X. Zhang, J.B. Buse, Z. Gu, Bioresponsive microneedles with a sheath structure for H₂O₂ and pH cascade-triggered insulin delivery, *Small* 14 (2018) 1704181.
- [171] H. Thabit, M. Tauschmann, J.M. Allen, L. Leelarathna, S. Hartnell, M.E. Wilinska, C.L. Acerini, S. Dellweg, C. Benesch, L. Heinemann, Home use of an artificial beta cell in type 1 diabetes, *N. Engl. J. Med.* 373 (2015) 2129–2140.
- [172] K. Burczak, T. Fujisato, M. Hatada, Y. Ikada, Protein permeation through poly(vinyl alcohol) hydrogel membranes, *Biomaterials* 15 (1994) 231–238.
- [173] C.R. Gordijo, K. Koulajian, A.J. Shuhendler, L.D. Bonifacio, H.Y. Huang, S. Chiang, G.A. Ozin, A. Giacca, X.Y. Wu, Nanotechnology-enabled closed loop insulin delivery device: *In vitro* and *in vivo* evaluation of glucose-regulated insulin release for diabetes control, *Adv. Funct. Mater.* 21 (2011) 73–82.
- [174] E. Ruel-Gariepy, J.-C. Leroux, *In situ*-forming hydrogels—review of temperature-sensitive systems, *Eur. J. Pharm. Biopharm.* 58 (2004) 409–426.
- [175] R.A. Siegel, Stimuli sensitive polymers and self regulated drug delivery systems: a very partial review, *J. Control. Release* 190 (2014) 337–351.
- [176] T. Sahota, P. Tomlins, M.J. Taylor, Long-term stability of glucose responsive dextran methacrylate-concanavalin A methacrylamide gels as part of an implantable artificial pancreas, *Int. J. Polym. Mater. Polym. Biomater.* 64 (2015) 946–954.
- [177] M.K. Chu, C.R. Gordijo, J. Li, A.Z. Abbasi, A. Giacca, O. Plettenburg, X.Y. Wu, *In vivo* performance and biocompatibility of a subcutaneous implant for real-time glucose-responsive insulin delivery, *Diabetes Technol. Ther.* 17 (2015) 255–267.
- [178] J. Liang, Y. Ma, S. Sims, L. Wu, A patterned porous polymer film for localized capture of insulin and glucose-responsive release, *J. Mater. Chem. B* 3 (2015) 1281–1288.
- [179] A. GhavamiNejad, J. Li, B. Lu, L. Zhou, L. Lam, A. Giacca, X.Y. Wu, Glucose-responsive composite microneedle patch for hypoglycemia-triggered delivery of native glucagon, *Adv. Mater.* 1901051 (2019).
- [180] J. Yu, Y. Zhang, W. Sun, A.R. Kahkoska, J. Wang, J.B. Buse, Z. Gu, Insulin-responsive glucagon delivery for prevention of hypoglycemia, *Small* 13 (2017) 1603028.
- [181] C.Y. Wong, H. Al-Salami, C.R. Dass, Microparticles, microcapsules and microspheres: a review of recent developments and prospects for oral delivery of insulin, *Int. J. Pharm.* 537 (2018) 223–244.
- [182] L.-C. Tsai, C.-H. Chen, C.-W. Lin, Y.-C. Ho, F.-L. Mi, Development of multifunctional nanoparticles self-assembled from trimethyl chitosan and fucoidan for enhanced oral delivery of insulin, *Int. J. Biol. Macromol.* 126 (2019) 141–150.