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Development of Glucose-Responsive “Smart” Insulin Systems

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Abstract

Purpose of Review—The complexity of modern insulin-based therapy for Type I and Type II diabetes mellitus and the risks associated with excursions in blood-glucose concentration (hyperglycemia and hypoglycemia) have motivated the development of “smart insulin” technologies (glucose-responsive insulin; GRI). Such analogs or delivery systems are entities that provide insulin activity proportional to the glycemic state of the patient without external monitoring by the patient or healthcare provider. This review describes the relevant historical background to modern GRI technologies and highlights three distinct approaches: coupling of continuous glucose monitoring (CGM) to deliver devices (algorithm-based “closed-loop” systems), glucose-responsive polymer encapsulation of insulin and molecular modification of insulin itself.

Recent Findings—Recent advances in GRI research utilizing each of the three approaches are illustrated; these include newly developed algorithms for CGM-based insulin delivery systems, glucose-sensitive modifications of existing clinical analogs, newly-developed hypoxia-sensitive polymer matrices, and polymer-encapsulated, stem-cell-derived pancreatic β -cells.

Summary—Although GRI technologies have yet to be perfected, the recent advances across several scientific disciplines that are described in this review have provided a path towards their clinical implementation.

Keywords

Glucose-responsive insulin; Artificial Pancreas; Glucose-responsive polymers; Diabetes; Hypoglycemia

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Introduction

Insulin replacement therapy is integral to the treatment of Type I diabetes (T1DM) and often required in the treatment of Type II diabetes (T2DM). These distinct diseases are respectively caused by an absolute or relative lack of insulin (1). Insulin initiates the uptake of glucose within peripheral tissues by binding to its molecular target, the insulin receptor (IR) (2). This receptor tyrosine kinase in turn activates a complex set of intracellular signaling processes (3) leading to translocation of glucose transporter GLUT4 to the cell surface (4). Regulated secretion of insulin by pancreatic β -cells in response to increased interstitial glucose levels ordinarily enables the blood-glucose level (BGL) to be maintained within a narrow range, which in healthy individuals lies between 80 and 120 mg/dl (4.4-5.6 mM) with fasting values between 80 and 100 mg/dl (5).

Unlike pancreatic secretion of insulin, standard pharmacological administration of insulin is not regulated by an endogenous feedback mechanism. Even with carefully designed dosing regimens and use of modern insulin products (i.e., engineered basal and rapid-acting insulin analogs) and even in the context of strict dietary and lifestyle adherence by patients, patients with diabetes often experience periods of hyperglycemia or hypoglycemia (6). The chronic health risks associated with elevated mean glycemia (micro- and macrovascular disease, renal disease, retinopathy, and neuropathy) are insidious relative to the dramatic presentation of acute metabolic decompensation (diabetic ketoacidosis in T1DM and hyperglycemic coma in T2DM) or the immediate signs and symptoms of hypoglycemia (adrenergic and neuroglycopenic effects leading to anxiety, tremulousness, and altered mental status leading in severe cases to coma) (7, 8). Further, such acute events can trigger adverse cardiovascular events in the setting of preexisting vascular disease (9), and repeated severe hypoglycemic episodes can cause cumulative damage to the brain with cognitive decline (10). For these reasons avoidance of hypoglycemia often limits individual hemoglobin A_{1c} goals (an integrated indicator of mean glycemia over three months) and the rigor of current insulin treatment regimens.

A long-standing goal has been development of insulin delivery systems that would *co-optimize* treatment of hyperglycemia and prevention of hypoglycemia. Such systems would, like endogenous pancreatic β -cells, provide insulin action proportionate to the glycemic state of the patient. Accordingly, “smart insulin” systems (broadly designated *glucose-responsive insulin*; GRI) have attracted the attention of the research community for four decades (11, 12). Such systems are defined as a class of insulin-delivery devices or insulin formulations that provide insulin activity commensurate with the metabolic needs of the patient. Due to the clinical considerations summarized above, the development of GRI technology has been a strategic priority of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (13) and the Juvenile Diabetes Research Fund (JDRF) (14).

Our definition of GRI is based on the *conceptual goal of glucose-regulated delivery* rather than any particular mechanical or molecular embodiment. Because this goal is shared by closed-loop delivery systems (i.e., the “artificial pancreas” as based on CGM-coupled insulin pumps (15)) and because such systems are near regulatory approval, they provide a starting point for our discussion. This perspective may be unconventionally, but highlights

clinical goals as our ultimate objective. In the coming decade closed-loop systems are likely to provide the gold standard with respect to which the efficacy and safety of “smart” molecular strategies will be evaluated.

With this in mind, GRIs may be broadly classified as follows: (i) algorithm-based mechanical GRI systems (15) in accordance with the above definition; (ii) polymer-based systems in which insulin is encapsulated within a glucose-responsive polymeric matrix-based vesicle or hydrogel (16); or (iii) molecular GRI analog systems, which involve the introduction of a glucose-sensitive motif to the insulin molecule or its formulation, in either case conferring glucose-responsive changes to the bioavailability or activity of the hormone (17).

Mechanical GRIs

Mechanical GRI systems have three components: (i) a CGM providing real-time measurement of the interstitial glucose concentration; (ii) an insulin pump capable of receiving data from the CGM and (iii) a computer-encoded algorithm that predicts the appropriate dose of insulin for subcutaneous (SQ) injection (18, 19). Optimization of such systems has led to efforts to enhance the accuracy and precision of CGMs and the robustness of the control algorithms. Because changes in interstitial glucose concentration lag by ~20 minutes behind changes in BGL, because the insulin analog (once injected) may require 20-40 min for absorption into the blood stream and because the hormone (once in the blood stream) may exert biological effects lasting 3-4 hours, a critical feature of modern algorithms is prediction of future trends in BGL. The robustness of such predictive algorithms is likely to be enhanced by the development of “ultra-fast” pump insulin analog formulations that minimize any delay in SQ absorption and in the duration of target-cell signaling, once the insulin receptor is engaged (20).

Advances in engineering over the past decade have led to the creation of compact devices in which CGMs are integrated within algorithm-controlled insulin pumps capable of precise adjustments in SQ delivery rate (21). Further, through analysis of clinical data and implementation of rapid signal-processing techniques, hierarchical algorithms that optimize glycemic control while prioritizing patient safety have been developed (20 - 23). These algorithms include such parameters as insulin potency, time since previous bolus dose, rate of increase of BGL, heart rate, and temperature to create closed-loop insulin delivery systems designed to maintain euglycemia during a variety of conditions: extent of exercise or activities of daily life (24*-26**), fluctuations in ambient temperature (27*), changes in diet (28, 29), variation in insulin sensitivity (30), and amount of insulin already on-board (31). To more effectively prevent or treat hypoglycemia, more complex pumps have been developed to independently provide either insulin or its counter-regulatory hormone glucagon (26, 32-34). This dual-hormone algorithm triggers SQ injection of a stabilized glucagon formulation based on trends in CGM readings predicting a hypoglycemic excursion. Such a bihormonal AP has been shown to maintain BGL with the targeted range more efficiently (i.e., with fewer hyperglycemic excursions and reduced time in hypoglycemia) than conventional CGM-coupled insulin pumps (35, 36**).

Mechanical GRIs may be further streamlined by optimization of each of its components. CGMs may be improved, for example, through ultra-miniaturization of sensors and improvements in their accuracy (37*-39). Development of novel insulin analogs with more rapid onset of activity and shorter duration of action (relative to current insulin analog products Humalog®, Novolog®, and Apidra® (40-42)) could improve closed-loop systems by providing more predictable pharmacodynamic profiles and hence more accurate calculation of insulin doses. Examples of more rapid formulations are provided by Novolog-based FIAsp (a reformulation of insulin aspart to contain excipients that promote capillary absorption) or addition of the degradative enzyme hyaluronidase to wild-type insulin (rHuPH20; Halozyme, Inc.) (43, 44*, 45*); the latter approach is no longer being pursued.

Intraperitoneal (IP) delivery of insulin may also offer advantages within closed-loop systems. IP delivery leads to ultra-rapid absorption and, like native pancreatic secretion, provides first-pass treatment of the liver. These features have led to the development of intraperitoneal infusion (IPI) devices (46, 47) as implanted and refillable pumps (48). Despite their theoretical advantages and long-term reservoirs (up to three months), regulatory approval of IPIs in the United States has been withheld due to their susceptibility to catheter occlusion (49). Such occlusions are in part inflammatory, provoked by formation of immunogenic and pro-inflammatory amyloid fibrils (50, 51). In addition, fibrillation of insulin within the pump reservoir or catheter alters the flow properties of the solution and inactivates the hormone. Occlusive events limited the feasibility of routine IPI delivery in an otherwise encouraging clinical trial at U.S. Veterans Administration hospitals (52). Nonetheless, long-term experience with intraperitoneal insulin pumps has been documented in Montpellier, FR with use of a special surfactant-stabilized U-400 insulin formulation (Insuplant®; Sanofi); periodic in-hospital alkaline washes of the implanted reservoir are required to remove any fibrils or amyloidogenic seeds (49).

The challenge of insulin degradation in pumps, whether external (53) or implanted (50), may be addressed by development of ultra-stable, fibrillation-resistant insulin analogs. Two classes of insulin analogs have been shown to be refractory to fibrillation: single-chain insulins (SCIs) containing a foreshortened C domain (Fig. 1A) (54) and two-chain insulin analogs containing an engineered non-canonical disulfide bridge between the A and B chains (55) (Fig. 1B). In each case the altered topology (connectivity) of the polypeptide is thought to be incompatible with regular cross- β assembly, the general structural mechanism underlying formation of amyloid (56-58). Although such analogs have not been tested clinically, insertion of a non-canonical cystine is associated in rat studies with anomalously prolonged duration of activity on intravenous bolus injection (55); this would be an unfavorable feature of a pump insulin.

Polymer and Matrix-Based GRIs

Polymer- or matrix-based GRI systems rely on sequestration of native or derivatized insulin molecules within a matrix suitable for SQ injection. The matrix is designed to sense ambient glucose concentrations and release a proportional amount of insulin for systemic absorption. Three classes of glucose-sensitive motifs permit glucose-dependent insulin sequestration: (i) glucose-binding proteins (GBPs), a class that includes lectins like concanavalin A (ConA),

(ii) glucose oxidase (GoD), an enzyme that catalyzes oxidation of glucose to gluconic acid with release of a proton (hence lowering the pH), and (iii) phenylboronic acid (PBA; Fig. 2), which forms reversible ester linkages with diol-containing molecules, including glucose itself (Table 1).

Brownlee and Cerami created the first model GRI system: glycosylated insulin complexed with ConA (11, 12). This complex was intended to sequester insulin in the SQ space during normoglycemia and release the hormone during hyperglycemia via competition with ambient glucose molecules. Although the strategy was successful *in vitro*, competitive setpoint was above typical hyperglycemic concentrations (66). Later studies suggested that the immunogenicity and mitogenicity of ConA would impair clinical feasibility (64, 65).

Advances in materials science have allowed development of polymer-based GRI systems. In such systems insulin is encapsulated within polymer matrices as a “smart” SQ depot. Polymeric vesicles (polymerosomes) and micro- or nanoparticles encapsulate insulin in a controlled SQ environment that may mitigate foreign-body reactions or immune responses (Fig. 3A) (88, 89) Such nanosystems are more amenable to fine tuning than is ConA complexation (Fig. 4A).

Polymer-based GRIs can employ a variety of encapsulation chemistries. These include polyethylene glycol, poly-N-vinyl-pyrrolidone, and succinyl-amidophenyl-glucopyranoside (16, 91-93) or designed bio-molecules such as modified peptides or lipids (94*, 95*). Whereas the matrices are impermeable to insulin during normoglycemic or hypoglycemic conditions, their permeability may increase as a result of structural changes that cause swelling or increased water solubility of the polymer in response to an increase in interstitial glucose concentration (Fig. 3B). To this end, molecular glucose sensors (GBPs, GoD, and PBA) have been incorporated into such polymer scaffolds.

Several strategies have been tested to incorporate glucose sensors into polymer-based GRIs. These include insulin-encapsulating particles composed of matrices co-derivatized with PBA or other boronic acids (87, 96*, 97*), dot-immobilized carbon or glucose ester-based crosslinks (98**). Immobilized GBPs have also been exploited in conjunction with glucose-modified polymers. The latter strategy underlies SmartInsulin[®] as developed by SmartCells, Inc. (67, 68*). GoD has also been encapsulated in matrices that are chemically sensitive to H₂O₂, hypoxia, or decreases in local pH. Unlike PBA and GPB-based technologies, polymeric matrices in GoD-based systems isomerize (94*) or are chemically modified by redox reactions (95*) that occur as a consequence of GoD activity, resulting in increased water-permeability of the encapsulating polymer (Fig. 3C).

GoD-based polymerosomes were recently employed by Yu and colleagues (2017) (74**) to encapsulate insulin in self-assembling polymersomes composed of poly(ethylene glycol) (PEG) and polystyrene (as modified with 2-nitroimidazole through a thioether linker). In this elegant scheme, local hypoxia induced by GoD activity promoted reduction of 2-nitroimidazole into the more hydrophilic 2-aminoimidazole by SQ reductases, causing the encapsulating polymer to swell and become more water-permeable. The thioester linker served a dual purpose: to scavenge H₂O₂ (a byproduct of GoD activity) and in so doing,

through conversion of the thioester to a sulfone, further increase the permeability of the matrix. After *in vitro* confirmation of the mechanism of action, the polymersomes were integrated with cross-linked hyaluronic acid microneedle arrays for painless transcutaneous delivery (99). This system was able to lower blood-glucose levels in diabetic mice within 60 minutes and maintain adequate glycemic control for approximately five hours. Critically, the system did not induce hypoglycemia in mice pre-treated with insulin.

Challenges faced by polymer-based GRI technologies include limited particle stability (100) and suboptimal response rates: too slow in responding either to an increase or decrease in GBL (respectively leading to hyperglycemic or hypoglycemic excursions). Addressing these limitations has often yielded paradoxical results: sensitizing matrices to hyperglycemia, for example, can limit their ability to attenuate insulin release at low glucose levels. The latter problem can be exacerbated by matrix degradation, raising the risk of a severe hypoglycemic episode. (76, 101**)

Several groups have addressed the above challenges by creating GRI systems with multiple and complementary molecular mechanisms of glucose sensing and response. An example is provided by the combination of GoD, pH-sensitive matrices, and PBA modifications of the matrix or encapsulated insulin itself (83, 102). Such a nanotechnology may respond rapidly to GoD activity and also retard the release of insulin during normoglycemia through its interactions with diols in the polymer matrix (101**, 103, 104). Variation in particle size and permeable surface area (105) and addition of multiple layers of polymeric matrix (100) have also been shown to modulate glucose-response rates. In several micro- and nanogel systems insulin has been encapsulated in largely impermeable polymer matrices that contain only small patches (or “pores”) of glucose-responsive polymeric material. This strategy seeks to limit the maximal rate of glucose release (thus mitigating risk of extreme hypoglycemia) while maintaining a rapid response to hyperglycemia (76).

An alternative strategy for polymer-based GRI systems exploits the self-regulatory capability of cadaveric pancreatic β -cells or those cultured from pluripotent stem cells (SC- β) to create a glucose-responsive system (106). A major barrier to β -cell implantation is posed by the foreign-body response (FBR) (107). Encapsulation in polymeric matrices has long been investigated as a means to isolate implanted cells from such tissue reactions at the injection site (108).

Whereas several studies have shown the ability of polymer-encapsulated SC- β s to provide glycemic control in *immunosuppressed* diabetic animals (109, 110), the first example of a functional polymer-encapsulated SC- β GRI in an *immunocompetent* animal was shown by Vegas and colleagues in 2016 (111**). SC- β s were encapsulated in 1.5 mm microspheres composed of azole-modified alginate (triazolethiomorpholine dioxide; TMTD). This demonstrated that in diabetic mice such particles maintained euglycemia with no hypoglycemic episodes for 150 days. Further, the particles elicited only limited immune activation and FBR as assessed by analysis of immunological molecules adsorbed to the surface of extracted particles and of fibrosis-associated biomolecular markers (112). The large size of the implanted particles (relative to other polymer-based GRI devices) and the derivatization of the alginate matrix with imidazole were identified as key innovations

(111**). These modifications have not yet been shown to mitigate immune response in humans, however, potentially limiting clinical applicability (111**, 113).

Molecular GRIs

Molecular GRIs represent a novel class of molecules in which the insulin molecule or its formulation confers glucose-dependent activity or bioavailability. Present candidate technologies rely on sequestration of active insulin hormone within the SQ space or within the bloodstream (as inactive complexes) with enhanced release or activation only during hyperglycemia. Although such strategies remain in the early stages of development, the potential convenience and cost-effectiveness of molecular GRIs make them an attractive target for future analog design.

An early strategy employed an insulin-GoD fusion molecule (75). A cysteine-based linkage was broken as the enzyme oxidized glucose. Although proof of principle was obtained *in vitro*, the low K_m of GoD for glucose led to liberation of the hormone under hypoglycemic as well as hyperglycemic conditions (75). In addition, the release of hydrogen peroxide by GoD (as a byproduct of glucose oxidation) could damage surrounding tissues via generation of reactive oxygen species (ROS). In an unrelated approach, GoD-dependent changes in ambient pH were exploited by Kashyap and colleagues to modulate the solubility (and hence bioavailability) of insulin glargine, the active component of Lantus (114). Because this basal analog is insoluble at neutral pH but soluble under acidic conditions, GoD-mediated acidification (via production of gluconic acid) was predicted to increase the bioavailability of the insulin analog (115) (Fig. 4B). Although this mechanism-based approach showed promise *in vitro* and demonstrated some improvement in glycemic control in pilot animal studies, no comprehensive report has been published (77).

PBA was first described in the context of a GRI system by Hoeg-Jensen *et al.* (17). The group demonstrated that derivatives of PBA could be coupled to insulin without affecting its biological activity, in turn enabling the analog to bind diol-containing sequestering agents (Fig. 4C). The investigators further demonstrated that insulin analogs co-derivatized with PBA and polyols could form glucose-dependent multimeric complexes *in vitro* (Fig. 4D) (116) (90). This form of glucose responsiveness has been engineered within insulin degludec, a basal acylated insulin (117). To our knowledge, no *in vivo* results have been described.

Recent use of a PBA-based GRI was described by the Langer-Anderson groups at MIT (86). This study employed an acylated insulin analog (detemir, the active component of Levemir®; Novo-Nordisk). Insulin detemir contains a myristic acid coupled to a unique lysine (Lys^{B29}), which mediates binding to serum albumin and so provides a long-lived circulating depot. The MIT groups sought to derivatize the acyl tag with PBA so that its affinity for albumin would be glucose-responsive (Fig. 3E; (86). Although this goal was not achieved *in vitro*, several of the candidate GRI analogs nonetheless exhibited glucose-responsive biological activity in a peritoneal glucose-infusion assay in mice. Molecular mechanisms underlying these provocative findings were not defined.

A new avenue for molecular GRI design may be opened by recent crystallographic studies of insulin bound to fragments of the IR ectodomain (2, 118). Such studies have revealed a major change in the conformation of insulin on receptor binding (Fig. 5). It may be possible to exploit this mechanism to design a glucose-dependent conformational switch such that binding of the modified insulin to the IR is impaired under conditions of hypoglycemia. This notion represents an active area of research.

Clinical Significance and Conclusions

The development of safe and effective GRIs promises to enhance the health and quality of life of patients with T1DM and of patients with T2DM refractory to oral therapy. Strict glycemic control has been shown to retard or prevent microvascular complications in T1DM (119) and is likely to be beneficial in early stages of T2DM; (120-122). Aggressive insulin regimens nonetheless increase the acute and long-term risks of hypoglycemia (123-125). Of particular concern as patients with T1D live longer with current standards of medical management, recurring hypoglycemic episodes are associated with cognitive decline (8).

In summary GRI technology offers the hope to provide intensive glycemic control without increased risk of hypoglycemia and with less burden on patients (7). Whereas CGM-pump-based GRIs presently employ the most mature component technologies, recent innovations in matrix-based and molecular GRIs are elegant and suggest promising routes toward functional replacement of the pancreatic β -cell. We anticipate continuing progress in the coming years.

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References

1. Kahn S. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia*. 2003; 46(1):3–19. [PubMed: 12637977]
2. Menting JG, Yang Y, Chan SJ, Phillips NB, Smith BJ, Whittaker J, et al. A structural hinge in insulin enables its receptor engagement. *Proc Natl Acad Sci USA*. 2014; 111(33):1–48.
3. White MF, Kahn CR. The insulin signaling system. *J Biol Chem*. 1994; 269:1–4. [PubMed: 8276779]
4. Saltiel AR, Pessin JE. Insulin signaling pathways in time and space. *Trends Cell Biol*. 2002; 12:65–71. [PubMed: 11849969]
5. Petznick A. Insulin management of type 2 diabetes mellitus. *Am Fam Physician*. 2011; 84(2):183–90. [PubMed: 21766768]
6. Campbell MD, Walker M, Bracken RM, Turner D, Stevenson EJ, Gonzalez JT, et al. Insulin therapy and dietary adjustments to normalize glycemia and prevent nocturnal hypoglycemia after evening exercise in type 1 diabetes: a randomized controlled trial. *BMJ Open Diabetes Research and Care*. 2015; 3(1):e000085.

7. Beregszászi M, Tubiana-Rufi N, Benali K, Noël M, Bloch J, Czernichow P. Nocturnal hypoglycemia in children and adolescents with insulin-dependent diabetes mellitus: prevalence and risk factors. *The Journal of pediatrics*. 1997; 131(1):27–33. [PubMed: 9255188]
8. Cryer PE, Davis SN, Shannon H. Hypoglycemia in Diabetes. *Diabetes Care*. 2003; 26:1902–12. [PubMed: 12766131]
9. Snell-Bergeon JK, Wadwa RP. Hypoglycemia, diabetes, and cardiovascular disease. *Diabetes technology & therapeutics*. 2012; 14(S1):S-51–S-8. [PubMed: 22650225]
10. Cryer, PE. Hypoglycemia: pathophysiology, diagnosis, and treatment. Oxford University Press; USA: 1997.
11. Brownlee M, Cerami A. A glucose-controlled insulin-delivery system: semisynthetic insulin bound to lectin. *Science*. 1979 Dec 7; 206(4423):1190–1. [PubMed: 505005]
12. Zaykov AN, Mayer JP, DiMarchi RD. Pursuit of a perfect insulin. *Nature Reviews Drug Discovery*. 2016
13. NIDDK. Committee DMIC. Advances and emerging opportunities in diabetes research: a Strategic Planning report of the DMICC. Washington, D.C: 2011.
14. Insel RA, Deecher DC, Brewer J. Juvenile Diabetes Research Foundation: mission, strategy, and priorities. *Diabetes*. 2012; 61(1):30–5. [PubMed: 22187372]
15. Halvorson M, Carpenter S, Kaiserman K, Kaufman FR. A pilot trial in pediatrics with the sensor-augmented pump: combining real-time continuous glucose monitoring with the insulin pump. *The Journal of pediatrics*. 2007; 150(1):103–5. e1. [PubMed: 17188626]
16. Ravaine V, Ancla C, Catargi B. Chemically controlled closed-loop insulin delivery. *J Control Release*. 2008 Nov 24; 132(1):2–11. [PubMed: 18782593]
17. Hoeg-Jensen T, Ridderberg S, Havelund S, Schäffer L, Balschmidt P, Jonassen I, et al. Insulins with built-in glucose sensors for glucose responsive insulin release. *J Pept Sci*. 2005; 11(6):339–46. [PubMed: 15635658]
18. Renard E. Implantable closed loop glucose sensing and insulin delivery: the future for insulin pump therapy. *Curr Opin Pharmacol*. 2002; 2:708–16. [PubMed: 12482735]
19. Renard E, Costalat G, Bringer J. From external to implantable insulin pump, can we close the loop? *Diabetes Metab*. 2002; 28:2S19–2S25. [PubMed: 12442060]
20. Breton M, Farret A, Bruttomesso D, Anderson S, Magni L, Patek S, et al. Fully integrated artificial pancreas in type 1 diabetes: modular closed-loop glucose control maintains near normoglycemia. *Diabetes*. 2012 Sep; 61(9):2230–7. [PubMed: 22688340]
21. Ly TT, Roy A, Grosman B, Shin J, Campbell A, Monirabbasi S, et al. Day and night closed-loop control using the integrated Medtronic hybrid closed-loop system in type 1 diabetes at diabetes camp. *Diabetes Care*. 2015; 38(7):1205–11. [PubMed: 26049550]
22. Doyle FJ, Huyett LM, Lee JB, Zisser HC, Dassau E. Closed-loop artificial pancreas systems: engineering the algorithms. *Diabetes Care*. 2014; 37(5):1191–70. [PubMed: 24757226]
23. Pinsker JE, Lee JB, Dassau E, Seborg DE, Bradley PK, Gondhalekar R, et al. Randomized crossover comparison of personalized MPC and PID control algorithms for the artificial pancreas. *Diabetes Care*. 2016; 39(7):1135–42. [PubMed: 27289127]
- 24*. DeBoer MD, Chervinský DR, Topchyan K, Kovatchev BP, Francis GL, Breton MD. Heart rate informed artificial pancreas system enhances glycemic control during exercise in adolescents with T1D. *Pediatric diabetes*. 2016 A controlled clinical trial of 18 adolescent patients using a heart-rate-informed artificial pancreas showed marked reduction of time in hypoglycemia compared to those using a standard artificial pancreas.
25. Miller S, Nimri R, Atlas E, Grunberg EA, Phillip M. Automatic learning algorithm for the MD-logic artificial pancreas system. *Diabetes technology & therapeutics*. 2011; 13(10):983–90. [PubMed: 21774690]
- 26*. Jacobs PG, El Youssef J, Reddy R, Resalat N, Branigan D, Condon J, et al. Randomized trial of a dual-hormone artificial pancreas with dosing adjustment during exercise compared with no adjustment and sensor-augmented pump therapy. *Diabetes Obesity and Metabolism*. 2016; 18(11):1110–90. A controlled trial demonstrated that a bi-hormonal pancreas using standardized algorithms for dosing during exercise was able to reduce hypoglycemic episodes compared to manually adjusted artificial pancreas doses.

- 27*. Turksoy K, Monforti C, Park M, Griffith G, Quinn L, Cinar A. Use of Wearable Sensors and Biometric Variables in an Artificial Pancreas System. *Sensors*. 2017; 17(3):532. Describes the analysis of biometric data collected from exercising patients to determine which variables are most predictive of blood-glucose level.
28. Lee H, Buckingham BA, Wilson DM, Bequette BW. A closed-loop artificial pancreas using model predictive control and a sliding meal size estimator. *Journal of diabetes science and technology*. 2009; 3(5):1082–90. [PubMed: 20144421]
29. Lee H, Bequette BW. A closed-loop artificial pancreas based on model predictive control: Human-friendly identification and automatic meal disturbance rejection. *Biomedical Signal Processing and Control*. 2009; 4(4):347–54.
30. Steil G, Clark B, Kanderian S, Rebrin K. Modeling insulin action for development of a closed-loop artificial pancreas. *Diabetes technology & therapeutics*. 2005; 7(1):94–108. [PubMed: 15738707]
31. Ellingsen C, Dassau E, Zisser H, Grosman B, Percival MW, Jovanovi L, et al. Safety constraints in an artificial pancreatic β cell: an implementation of model predictive control with insulin on board. *Journal of diabetes science and technology*. 2009; 3(3):536–44. [PubMed: 20144293]
32. Bakhtiani PA, Caputo N, Castle JR, El Youssef J, Carroll JM, David LL, et al. A novel, stable, aqueous glucagon formulation using ferulic acid as an excipient. *Journal of diabetes science and technology*. 2014; 9(1):17–23. [PubMed: 25253164]
33. Haidar A, Legault L, Messier V, Mitre TM, Leroux C, Rabasa-Lhoret R. Comparison of dual-hormone artificial pancreas, single-hormone artificial pancreas, and conventional insulin pump therapy for glycaemic control in patients with type 1 diabetes: an open-label randomised controlled crossover trial. *The lancet Diabetes & endocrinology*. 2015; 3(1):17–26. [PubMed: 25434967]
34. Haidar A, Legault L, Matteau-Pelletier L, Messier V, Dallaire M, Ladouceur M, et al. Outpatient overnight glucose control with dual-hormone artificial pancreas, single-hormone artificial pancreas, or conventional insulin pump therapy in children and adolescents with type 1 diabetes: an open-label, randomised controlled trial. *The lancet Diabetes & endocrinology*. 2015; 3(8):595–604. [PubMed: 26066705]
35. Blauw H, Keith-Hynes P, Koops R, DeVries JH. A review of safety and design requirements of the artificial pancreas. *Annals of Biomedical Engineering*. 2016; 44(11):3158–72. [PubMed: 27352278]
- 36*. El-Khatib FH, Balliro C, Hillard MA, Magyar KL, Ekhlaspour L, Sinha M, et al. Home use of a bihormonal bionic pancreas versus insulin pump therapy in adults with type 1 diabetes: a multicentre randomised crossover trial. *The Lancet*. 2016 A large multi-center at-home study of bihormonal artificial pancreas that demonstrated the ability of the device to reduce periods of hypoglycemia and maintain target BGLs more efficiently than standard CGM insulin pumps.
- 37*. Ribet F, Stemme G, Roxhed N. Ultra-miniaturization of a planar amperometric sensor targeting continuous intradermal glucose monitoring. *Biosensors and Bioelectronics*. 2017; 90:577–83. Describes the development of an implantable, ultra-miniaturized CGM device that comprises an electrode and thin-layers of polymer membranes that is implanted into the dermis of a patient and can provide dynamic measurement of BGLs. [PubMed: 27825874]
38. Graf A, McAuley SA, Sims C, Ulloa J, Jenkins AJ, Voskanyan G, et al. Moving Toward a Unified Platform for Insulin Delivery and Sensing of Inputs Relevant to an Artificial Pancreas. *Journal of diabetes science and technology*. 2016 1932296816682762.
39. Thabit H, Hovorka R. Coming of age: the artificial pancreas for type 1 diabetes. *Diabetologia*. 2016; 59(9):1795–805. [PubMed: 27364997]
40. Loughheed WD, Zinman B, Strack TR, Janis LJ, Weymouth AB, Bernstein EA, et al. Stability of insulin lispro in insulin infusion systems. *Diabetes Care*. 1997; 20:1061–5. [PubMed: 9203437]
41. Mudaliar SR, Lindberg FA, Joyce M, Beerdsen P, Strange P, Lin A, et al. Insulin Aspart (B28 Asp-Insulin): A Fast-Acting Analog of Human Insulin. *Diabetes Care*. 1999; 22:1501–6. [PubMed: 10480516]
42. Becker RH, Frick AD, Burger F, Potgieter JH, Scholtz H. Insulin glulisine, a new rapid-acting insulin analogue, displays a rapid time-action profile in obese non-diabetic subjects. *Exp Clin Endocrinol Diabetes*. 2005; 113:435–43. [PubMed: 16151977]

43. Vaughn DE, Yocum RC, Muchmore DB, Sugarman BJ, Vick AM, Bilinsky IP, et al. Accelerated pharmacokinetics and glucodynamics of prandial insulins injected with recombinant human hyaluronidase. *Diabetes technology & therapeutics*. 2009; 11(6):345–52. [PubMed: 19459762]
- 44*. Heise T, Hövelmann U, Zijlstra E, Stender-Petersen K, Jacobsen JB, Haahr H. A Comparison of Pharmacokinetic and Pharmacodynamic Properties Between Faster-Acting Insulin Aspart and Insulin Aspart in Elderly Subjects with Type 1 Diabetes Mellitus. *Drugs & Aging*. 2017; 34(1): 29–38. This study provides the clinical corroboration of pharmacodynamic and pharmacokinetic data regarding FIAsp. [PubMed: 27873152]
- 45*. Heise T, Pieber TR, Danne T, Erichsen L, Haahr H. A pooled analysis of clinical pharmacology trials investigating the pharmacokinetic and pharmacodynamic characteristics of fast-acting insulin aspart in adults with type 1 diabetes. *Clinical Pharmacokinetics*. 2017:1–9. A description of the new formulation of insulin aspart, called FIAsp, and its increased onset of action and blood-glucose recovery after injection. [PubMed: 27314261]
46. Schade DS, Eaton RP, Friedman JE, Spencer WJ. Normalization of plasma insulin profiles with intraperitoneal insulin in diabetic man. *Diabetologia*. 1980; 19:35–9. [PubMed: 6993266]
47. Schade DS, Eaton P. The peritoneum—a potential insulin delivery route for a mechanical pancreas. *Diabetes Care*. 1980; 3(2):229–34. [PubMed: 6993133]
48. Irsigler K, Kritiz H, Hagmüller G, Franetzki M, Prestele K, Thurow H, et al. Long-term continuous intraperitoneal insulin infusion with an implanted remote-controlled insulin infusion device. *Diabetes*. 1981; 30(12):1072–5. [PubMed: 7308587]
49. Renard E, Boutleau S, Jacques-Apostol D, lauton D, Gibert-Boulet F, Costalat G, et al. Insulin underdelivery from implanted pumps using peritoneal route. Determinant role of insulin pump compatibility. *Diabetes Care*. 1996; 19:812–7. [PubMed: 8842596]
50. Bally L, Thabit H, Hovorka R. Finding the right route for insulin delivery—an overview of implantable pump therapy. Expert opinion on drug delivery. 2017 just-accepted.
51. Jeandidier N, Boivin S, Sapin R, Rosart-Ortega F, Uring-Lambert B, Reville P, et al. Immunogenicity of intraperitoneal insulin infusion using programmable implantable devices. *Diabetologia*. 1995; 38:577–84. [PubMed: 7489841]
52. Saudek CD, Duckworth WC, Giobbie-Hurder A, Henderson WG, Henry RR, Kelley DE, et al. Implantable insulin pump vs multiple-dose insulin for non-insulin-dependent diabetes mellitus: a randomized clinical trial. Department of Veterans Affairs Implantable Insulin Pump Study Group. *JAMA*. 1996; 276:1322–7. [PubMed: 8861991]
53. Cobelli C, Renard E, Kovatchev BP, Keith-Hynes P, Ben Brahim N, Place J, et al. Pilot studies of wearable outpatient artificial pancreas in type 1 diabetes. *Diabetes Care*. 2012 Sep; 35(9):e65–7. [PubMed: 22923687]
54. Hua QX, Nakagawa SH, Jia W, Huang K, Phillips NB, Hu SQ, et al. Design of an active ultrastable single-chain insulin analog: synthesis, structure, and therapeutic implications. *J Biol Chem*. 2008 May 23; 283:14703–16. [PubMed: 18332129]
55. Vinther TN, Norrman M, Ribel U, Huus K, Schlein M, Steensgaard DB, et al. Insulin analog with additional disulfide bond has increased stability and preserved activity. *Protein Sci*. 2013; 22(3): 296–305. [PubMed: 23281053]
56. Tycko R. Solid-state NMR studies of amyloid fibril structure. *Annu Rev Phys Chem*. 2011 May. 62:279–99. [PubMed: 21219138]
57. Tycko R, Ishii Y. Constraints on supramolecular structure in amyloid fibrils from two-dimensional solid-state NMR spectroscopy with uniform isotopic labeling. *J Am Chem Soc*. 2003; 125:6606–7. [PubMed: 12769550]
58. Dobson CM. Protein folding and misfolding. *Nature*. 2003; 426:884–90. [PubMed: 14685248]
59. Springsteen G, Wang B. A detailed examination of boronic acid–diol complexation. *Tetrahedron*. 2002; 58(26):5291–300.
60. Guo Z, Shin I, Yoon J. Recognition and sensing of various species using boronic acid derivatives. *Chemical Communications*. 2012; 48(48):5956–67. [PubMed: 22576233]
61. Weis WI, Drickamer K. Structural basis of lectin-carbohydrate recognition. Annual review of biochemistry. 1996; 65(1):441–73.

62. Paulová M, Tichá M, Entlicher G, Košťál J, Kocourek J. Relationship between red blood cell agglutination and polysaccharide precipitation by phytohemagglutinin of *Pisum sativum* L. *FEBS letters*. 1970; 9(6):345–7. [PubMed: 11947711]
63. Drickamer K, Taylor ME. Biology of animal lectins. *Annual review of cell biology*. 1993; 9(1): 237–64.
64. Ballerstadt R, Evans C, McNichols R, Gowda A. Concanavalin A for *in vivo* glucose sensing: a biotoxicity review. *Biosens Bioelectron*. 2006 Aug 15; 22(2):275–84. [PubMed: 16488598]
65. Coutinho A, Larsson EL, Grönvik KO, Andersson J. Studies on T lymphocyte activation II. The target cells for concanavalin A-induced growth factors. *European journal of immunology*. 1979; 9(8):587–92. [PubMed: 159185]
66. Brownlee M, Cerami A. Glycosylated insulin complexed to Concanavalin A. Biochemical basis for a closed-loop insulin delivery system. *Diabetes*. 1983 Jun; 32(6):499–504. [PubMed: 6354778]
67. Zion TC, Tsang HH, Ying JY. Glucose-sensitive nanoparticles for controlled insulin delivery. 2003
- 68*. Wang C, Ye Y, Sun W, Yu J, Wang J, Lawrence DS, et al. Red Blood Cells for Glucose-Responsive Insulin Delivery. *Advanced Materials*. 2017 A GBP-based nanoparticle GRI in which nanoparticles were coated with glucose carriers, such as GLUT1, found on red blood cell membranes (mRBC) to sequester glycosylated insulin.
69. Wilson R, Turner A. Glucose oxidase: an ideal enzyme. *Biosensors and Bioelectronics*. 1992; 7(3): 165–85.
70. Janssen FW, Ruelius HW. Carbohydrate oxidase, a novel enzyme from *Polyporus obtusus*: II. Specificity and characterization of reaction products. *Biochimica et Biophysica Acta (BBA)-Enzymology*. 1968; 167(3):501–10.
71. Ngo TT, Lenhoff HM. Amperometric assay for collagenase. *Applied biochemistry and biotechnology*. 1983; 8(5):407–14. [PubMed: 6089662]
72. Leatham A, Brooks S. Enzyme binding to detect carbohydrate expression in tissue sections. *The Histochemical Journal*. 1987; 19(8):405–12. [PubMed: 3123427]
73. Coulthard C, Michaelis R, Short W, Sykes G, Skrimshire G, Standfast A, et al. Notatin: an anti-bacterial glucose-aerodehydrogenase from *Penicillium notatum* Westling and *Penicillium resticulosum* sp. nov. *Biochemical Journal*. 1945; 39(1):24. [PubMed: 16747849]
- 74*. Yu J, Qian C, Zhang Y, Cui Z, Zhu Y, Shen Q, et al. Hypoxia and H₂O₂ Dual-Sensitive Vesicles for Enhanced Glucose-Responsive Insulin Delivery. *Nano Letters*. 2017 Describes a dual-sensitive polymer GRI in which GoD is co-encapsulated with insulin within a matrix that is sensitive to hypoxia and H₂O₂. The scaffold provided release of insulin proportional to ambient glucose concentrations and was able to maintain adequate BGLs in rats.
75. Ito Y, Imanishi Y. Protein device for glucose-sensitive release of insulin. *ACS Publications*. 1994
76. Gu Z, Dang TT, Ma M, Tang BC, Cheng H, Jiang S, et al. Glucose-responsive microgels integrated with enzyme nanocapsules for closed-loop insulin delivery. *ACS Nano*. 2013; 7(8):6758–66. [PubMed: 23834678]
77. Kasyhap, N.Pohl, R.Bruen, K.Leone, E., Steiner, S., editors. “Smart” Basal Insulin Formulation that Releases Insulin in Response to Blood Glucose Concentrations of Diabetic Swine. 2010 Annual Meeting- Controlled Release Society; Portland, OR. 2010.
78. Friedman S, Pizer R. Mechanism of the complexation of phenylboronic acid with oxalic acid. Reaction which requires ligand donor atom protonation. *Journal of the American Chemical Society*. 1975; 97(21):6059–62.
79. Yan J, Springsteen G, Deeter S, Wang B. The relationship among pKa, pH, and binding constants in the interactions between boronic acids and diols—it is not as simple as it appears. *Tetrahedron*. 2004; 60:11205–9.
80. Nicholls MP, Paul PK. Structures of carbohydrate–boronic acid complexes determined by NMR and molecular modelling in aqueous alkaline media. *Organic & biomolecular chemistry*. 2004; 2(10):1434–41. [PubMed: 15136798]
81. Yilmaz MD, Xue M, Ambrogio MW, Buyukcakil O, Wu Y, Frascioni M, et al. Sugar and pH dual-responsive mesoporous silica nanocontainers based on competitive binding mechanisms. *Nanoscale*. 2015; 7(3):1067–72. [PubMed: 25475070]

82. Matsumoto A, Ishii T, Nishida J, Matsumoto H, Kataoka K, Miyahara Y. A Synthetic Approach Toward a Self-Regulated Insulin Delivery System. *Angewandte Chemie*. 2012; 124(9):2166–70.
83. Wang Y, Zhang X, Han Y, Cheng C, Li C. pH-and glucose-sensitive glycopolymer nanoparticles based on phenylboronic acid for triggered release of insulin. *Carbohydrate polymers*. 2012; 89(1): 124–31. [PubMed: 24750613]
84. Yang W, Gao X, Wang B. Boronic acid compounds as potential pharmaceutical agents. *Medicinal Research Reviews*. 2003; 23:346–268. [PubMed: 12647314]
85. Deshayes S, Cabral H, Ishii T, Miura Y, Kobayashi S, Yamashita T, et al. Phenylboronic acid-installed polymeric micelles for targeting sialylated epitopes in solid tumors. *Journal of the American Chemical Society*. 2013; 135(41):15501–7. [PubMed: 24028269]
86. Chou DH-C, Webber MJ, Tang BC, Lin AB, Thapa LS, Deng D, et al. Glucose-responsive insulin activity by covalent modification with aliphatic phenylboronic acid conjugates. *Proc Natl Acad Sci USA*. Feb 24; 2015 112(8):2401–6. [PubMed: 25675515]
87. Jin X, Zhang X, Wu Z, Teng D, Zhang X, Wang Y, et al. Amphiphilic random glycopolymer based on phenylboronic acid: synthesis, characterization, and potential as glucose-sensitive matrix. *Biomacromolecules*. 2009; 10(6):1337–45. [PubMed: 19397257]
88. Lopes JR, Santos G, Barata P, Oliveira R, Lopes CM. Physical and chemical stimuli-responsive drug delivery systems: targeted delivery and main routes of administration. *Current pharmaceutical design*. 2013; 19(41):7169–84. [PubMed: 23489197]
89. Frost GI. Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration. *Expert opinion on drug delivery*. 2007; 4(4):427–40. [PubMed: 17683255]
90. Hoeg-Jensen, T., Havelund, S., Markussen, J., Ostergaard, S., Ridderberg, S., Balschmidt, P., et al. Novo Nordisk A/S, assignee. Glucose dependent release of insulin from glucose sensing insulin derivatives. United States: 2008. inventors
91. Podual K, Doyle F, Peppas NA. Modeling of water transport in and release from glucose-sensitive swelling-controlled release systems based on poly (diethylaminoethyl methacrylate-g-ethylene glycol). *Industrial & engineering chemistry research*. 2004; 43(23):7500–12.
92. Lee YM, Kim SH, Cho CS. Synthesis and swelling characteristics of pH and thermoresponsive interpenetrating polymer network hydrogel composed of poly (vinyl alcohol) and poly (acrylic acid). *Journal of applied polymer science*. 1996; 62(2):301–11.
93. Kitano S, Koyama Y, Kataoka K, Okano T, Sakurai Y. A novel drug delivery system utilizing a glucose responsive polymer complex between poly (vinyl alcohol) and poly (N-vinyl-2-pyrrolidone) with a phenylboronic acid moiety. *Journal of controlled release*. 1992; 19(1-3):161–70.
- 94*. Li X, Fu M, Wu J, Zhang C, Deng X, Dhinakar A, et al. pH-sensitive peptide hydrogel for glucose-responsive insulin delivery. *Acta biomaterialia*. 2017 Describes the development of a peptide-based pH-sensitive hydrogel that encapsulates insulin, GoD, and catalase. The self-assembling peptides alter their conformation in response to changes in pH to release insulin.
- 95*. Anirudhan T, Nair AS, Nair SS. Enzyme coated beta-cyclodextrin for effective adsorption and glucose-responsive closed-loop insulin delivery. *International journal of biological macromolecules*. 2016; 91:818–27. Describes the development of a pH-sensitive hydrogel particle that is coated with GoD and catalase and swells in response to increased glucose levels. [PubMed: 27296445]
- 96*. Wang Y, Huang F, Sun Y, Gao M, Chai Z. Development of shell cross-linked nanoparticles based on boronic acid-related reactions for self-regulated insulin delivery. *Journal of Biomaterials Science Polymer Edition*. 2017; 28(1):93–106. A recent design of a PBA-based nanoparticle GRI that included an efficient synthesis method of standardized, stable insulin-encapsulating particles that released insulin *in vitro* at physiologically relevant glucose concentrations. [PubMed: 27719550]
- 97*. Wang H, Yi J, Yu Y, Zhou S. NIR upconversion fluorescence glucose sensing and glucose-responsive insulin release of carbon dot-immobilized hybrid microgels at physiological pH. *Nanoscale*. 2017; 9(2):509–16. A PBA-containing polymer-based GRI was synthesized with carbon dots, which are small molecules that serve as fluorescent reporters of glucose-responsive conformational changes and insulin release. [PubMed: 27942663]

- 98**. Dong Y, Wang W, Veisoh O, Appel EA, Xue K, Webber MJ, et al. Injectable and Glucose-Responsive Hydrogels Based on Boronic Acid–Glucose Complexation. *Langmuir*. 2016; 32(34): 8743–7. Investigation of ratios of boronic acid moieties to polymer-scaffold material that yield the most stable, glucose responsive, and reversibly permeable hydrogel. [PubMed: 27455412]
99. Xie S, Li Z, Yu Z. Microneedles for transdermal delivery of insulin. *Journal of Drug Delivery Science and Technology*. 2015; 28:11–7.
100. Shi D, Ran M, Zhang L, Huang H, Li X, Chen M, et al. Fabrication of biobased polyelectrolyte capsules and their application for glucose-triggered insulin delivery. *ACS applied materials & interfaces*. 2016; 8(22):13688–97. [PubMed: 27210795]
- 101**. Yesilyurt V, Webber MJ, Appel EA, Godwin C, Langer R, Anderson DG. Injectable Self-Healing Glucose-Responsive Hydrogels with pH-Regulated Mechanical Properties. *Advanced Materials*. 2016; 28(1):86–91. Describes a dynamic hydrogel created with three different PBA derivatives and *cis*-diol groups. The resulting hydrogel was stable and could swell reversibly in response to increased glucose concentrations. The gel also showed blunted response to changes in pH. [PubMed: 26540021]
102. Li J, Yang L, Fan X, Zhang J, Wang F, Wang Z. Temperature and Glucose Dual-Responsive Carriers Bearing Poly (N-Isopropylacrylamide) and Phenylboronic Acid for Insulin Controlled Release: A Review. *International Journal of Polymeric Materials and Polymeric Biomaterials*. 2017 just-accepted.
103. Matsumoto A, Yoshida R, Kataoka K. Glucose-responsive polymer gel bearing phenylborate derivative as a glucose-sensing moiety operating at the physiological pH. *Biomacromolecules*. 2004; 5(3):1038–45. [PubMed: 15132698]
104. Kikuchi A, Suzuki K, Okabayashi O, Hoshino H, Kataoka K, Sakurai Y, et al. Glucose-sensing electrode coated with polymer complex gel containing phenylboronic acid. *Analytical chemistry*. 1996; 68(5):823–8. [PubMed: 21619178]
105. Sun L, Zhang X, Zheng C, Wu Z, Li C. A pH gated, glucose-sensitive nanoparticle based on worm-like mesoporous silica for controlled insulin release. *The journal of physical chemistry B*. 2013; 117(14):3852–60. [PubMed: 23517533]
106. Hirshberg B. Lessons learned from the international trial of the edmonton protocol for islet transplantation. *Current diabetes reports*. 2007; 7(4):301–3. [PubMed: 17686407]
107. Robertson RP. Islet transplantation as a treatment for diabetes—a work in progress. *New England Journal of Medicine*. 2004; 350(7):694–705. [PubMed: 14960745]
108. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science*. 1980; 210(4472):908–10. [PubMed: 6776628]
109. Lum Z-P, Tai IT, Krestow M, Norton J, Vacek I, Sun AM. Prolonged reversal of diabetic state in NOD mice by xenografts of microencapsulated rat islets. *Diabetes*. 1991; 40(11):1511–6. [PubMed: 1936609]
110. Schneider S, Feilen PJ, Brunnenmeier F, Minnemann T, Zimmermann H, Zimmermann U, et al. Long-term graft function of adult rat and human islets encapsulated in novel alginate-based microcapsules after transplantation in immunocompetent diabetic mice. *Diabetes*. 2005; 54(3): 687–93. [PubMed: 15734844]
- 111**. Vegas AJ, Veisoh O, Gürtler M, Millman JR, Pagliuca FW, Bader AR, et al. Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nature medicine*. 2016 Describes a GRI system in which stem-cell derived pancreatic β -cells were encapsulated within an alginate derivatized TMTD polymer matrix. The GRI was able to maintain euglycemia in mice with reduced immune response. This was the first demonstration of a polymer-encapsulated β -cell transplantation in immunocompetent animals.
112. Kolb M, Bonniaud P, Galt T, Sime PJ, Kelly MM, Margetts PJ, et al. Differences in the fibrogenic response after transfer of active transforming growth factor- β 1 gene to lungs of “fibrosis-prone” and “fibrosis-resistant” mouse strains. *American journal of respiratory cell and molecular biology*. 2002; 27(2):141–50. [PubMed: 12151305]
113. Elliott, R.Escobar, L.Tan, P.Garkavenko, O.Calafiore, R.Basta, P., et al., editors. *Transplantation proceedings*. Elsevier; 2005. Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates.

114. Hilgenfeld R, Seipke G, Berchtold H, Owens DR. The Evolution of Insulin Glargine and its Continuing Contribution to Diabetes Care. *Drugs*. 2014; 74(8):911–27. [PubMed: 24866023]
115. Gillies PS, Figgitt DP, Lamb HM. Insulin glargine. *Drugs*. 2000; 59(2):253–60. [PubMed: 10730548]
116. Hoeg-Jensen, T., Havelund, S., Markussen, J. Novo Nordisk A/S assignee. Glucose-dependent insulins. United States: 2008. inventors
117. Jonassen I, Havelund S, Hoeg-Jensen T, Steensgaard DB, Wahlund PO, Ribel U. Design of the novel protraction mechanism of insulin degludec, an ultra-long-acting basal insulin. *Pharm Res*. 2012 Aug; 29(8):2104–14. [PubMed: 22485010]
118. Menting JG, Whittaker J, Margetts MB, Whittaker LJ, Kong GK, Smith BJ, et al. How insulin engages its primary binding site on the insulin receptor. *Nature*. 2013 Jan 10; 493(7431):241–5. [PubMed: 23302862]
119. DCCT. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med*. 1993 Sep 30; 329(14):977–86. [PubMed: 8366922]
120. UKPDS UPDSG. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998 Sep 12; 352(9131):837–53. [PubMed: 9742976]
121. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2008 Jun 12; 358(24):2560–72. [PubMed: 18539916]
122. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med*. 2009 Jan 8; 360(2):129–39. [PubMed: 19092145]
123. Control D, Group CTR. Hypoglycemia in the diabetes control and complications trial. *Diabetes*. 1997; 46(2):271–86. [PubMed: 9000705]
124. Group DR. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. *The American journal of medicine*. 1991; 90(4):450–9. [PubMed: 2012085]
125. Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, et al. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med*. 2008 Jun 12; 358(24):2545–59. [PubMed: 18539917]

Key Points

- Although strict glycemic control is beneficial to patients with T1DM and in a subset of patients with T2DM, aggressive glycemic targets increase the risk of hypoglycemia, which has severe acute and long-term sequelae.
- Glucose-responsive insulin (GRI) technologies represent a class of insulin-delivery devices or insulin formulations that provide insulin activity commensurate with the metabolic needs of the patient, effectively limiting hyperglycemia while simultaneously preventing hypoglycemic episodes.
- Three major classes of GRIs have been developed: (i) mechanical GRIs, which constitute continuous glucose monitors and insulin pumps integrated by hierarchical control algorithms, (ii) polymer-based GRIs in which insulin is encapsulated within a glucose-sensitive polymeric matrix, and (iii) molecular GRIs, in which the insulin molecule or its formulation have intrinsic glucose-responsive activity.

Figure 1. Cartoon of structure of ultra-stable insulin analogs

(A) Single chain insulin (SCI) contains the native A-domain (dark gray) and B-domain (light gray) and the three native disulfide linkages (yellow) of insulin. In addition, SCI analogs contain a foreshortened “C-domain” (6-8 amino acids in length) (orange) that connects the C-terminal strand of the B-domain (green) to the N-terminus of the A-domain. This domain dampens the conformational fluctuations of the molecule thus increasing its thermodynamic stability and rendering it resistant to fibrillation (54). (PDB ID: 2LWZ) (B) 4SS-insulin is a two-chain insulin analog (A-chain, dark gray, B-chain, light gray) that contains an engineered fourth disulfide linkage (red asterisk) between the A- and B-chains in addition to its three native disulfide linkages (yellow). The analog was reported to be resistant to fibrillation and have increased temperature stability (55). (PDB ID: 4EFX)

Figure 2. Schematic of phenylboronic acid-monosaccharide complexation

Phenylboronic acid (PBA) comprises an aryl ring containing a boronic acid substituent group. When the boronic acid moiety is ionized to phenylboronate, the group may form reversible ester bonds with *cis*-diols, including those found on monosaccharides (59). The pK_a of unmodified PBA is 8.0 and it has an affinity for glucose of approximately 10 mM. Both of these properties may be modulated by introducing electron-withdrawing substituents to the aryl ring (red circle marked with “X”) (60). A variety of groups, including nitro-, fluoro-, carboxyl, and sulfone groups have been shown to lower pK_a of PBA to below physiological pH (7.4), affinities of these derivatives for glucose range from 8-14 mM (17).

Figure 3. Cartoon representation of polymer-based GRIs

(A) Insulin is encapsulated within a polymeric matrix in large implants, transdermal patches, or micro- or nanoparticles. The native or derivatized insulin analog is typically sequestered within a water-containing cavity within the hydrophobic, water impermeable polymer matrix. Such matrices are compact during hypo- or euglycemia, but swell during hypoglycemia to release sequestered insulin (B). Several different methods have been used to create glucose-responsive polymers (C). (Top panels, C) PBA (green hexagon) and glucose have been incorporated into polymeric scaffolds to maintain the compactness of the matrix. This interaction is competitively disfavored as ambient glucose concentrations rise, causing the polymer to swell (top panels, C). (Middle panels, C) Immobilized or co-encapsulated GBPs (red shapes) have been used as agents that stabilize the compactness of polymeric matrices in a glucose-dependent fashion. (Bottom panels, C) Matrices that are sensitive to the byproducts of co-encapsulated or immobilized GoD (red polygon), gluconic acid and H_2O_2 , along with the resulting decrease in pH and local hypoxia, have been developed. Oftentimes, polymeric matrices will undergo conformational changes resulting from changes in the protonation state of components (blue circles, red lines) of the scaffold or from reactions that are catalyzed by the byproducts of GoD.

Figure 4. Cartoon representation of derivatization of the insulin molecule in GRI technology

The insulin hormone comprises two polypeptide chains that are designated “A” and “B” and are connected by two disulfide linkages spanning residues A7 and B7 and A20 and B19. Brownlee, *et al* created a molecular GRI by coupling mono- and disaccharides to the N-termini of one or both polypeptide chains of insulin (red). These analogs were bound to ConA (red star) before administration. ConA was expected to sequester the insulin in the SQ space during euglycemic conditions (left, A) and allow it to be liberated by competitive binding of ambient glucose (blue hexagons) during hyperglycemia (right, A) (11) (66). (B) Kashyap and colleagues created a GRI system using the clinical analog insulin glargine. Glargine contains an addition of two arginine residues at the C-terminus of its B-chain and a substitution of Asn^{A21} for glycine (77). These modifications shift the isoelectric point of the protein to near physiological conditions causing it to form precipitates after SQ injection. In this GRI system glargine was co-injected with GoD (red polygon), which was expected to lower the local pH by oxidizing glucose to gluconic acid at a rate proportional to the glycemic state of the patient increasing the solubility and, hence, the bioavailability of glargine. (C-E) A number of groups have modified the ϵ -amino group of Lys^{B29} with PBA derivatives (green hexagons) to create GRI systems. (C) Hoeg-Jensen and colleagues directly coupled Lys^{B29} with PBA derivatives and demonstrated the ability of the analog to couple to diol-containing polymer carriers (orange rectangles with red circles) in a glucose-dependent fashion (17). (D) The same group also derivatized residue B29 with a molecule containing a PBA and a polyol group (black lines with red circles) and demonstrated the ability of the analog to form multi-hexameric complexes *in vitro* that could dissociate in a glucose-dependent fashion (90). (E) Most recently, Chou and colleagues created a GRI that contained a PBA derivative coupled via a fatty acyl linker (black, jagged line with green hexagon) to Lys^{B29}, it was hypothesized that this analog would bind to albumin (blue oval) under normoglycemic conditions and become liberated during hyperglycemia as free glucose complexed with the PBA molecule and decreased the affinity of the analog for

albumin (86). Although this analog did show glucose-responsiveness in a mouse model, glucose-dependent association or dissociation from albumin was not confirmed.

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Figure 5. Structural representation of insulin IR binding

(A) The ectodomain of insulin receptor (IR) (anterior monomer of homodimer shown in color), the molecular target of insulin. The L1 and αCT (purple) domains of IR comprise the insulin binding site. (B) In its "closed" or storage conformation, insulin cannot engage its binding site due to a steric clash between αCT (purple) and the C-terminal B-chain of insulin (brown). (C) To engage its primary binding site, insulin must undergo a conformational change in which the C-terminal B-chain detaches from the hydrophobic core of the globular protein via unfolding of a β-turn spanning residues B20-B23 (red box in B and C). (D) The detachment of the C-terminal B-chain from the hydrophobic core of insulin allows the molecule to intercalate between the L1 and αCT domains and occupy its binding site.

Table 1**Glucose-Sensitive Motifs Employed in GRI Systems**

Motif and Description	Examples	Advantages	Disadvantages	Implementation
Glucose Binding Proteins Bind glucose and oligosaccharides	wheat germ agglutinin(WGA) concanavalin A(ConA) GLUT1	<ul style="list-style-type: none"> Naturally occurring (61) Scalable Manufacture(62) Structurally robust (62) 	<ul style="list-style-type: none"> Non-physiological glucose affinity(63) Immunogenicity (64) ConA mitogenic (65) 	<ul style="list-style-type: none"> Pre-bound complexes with insulin(66) Immobilized in hydrogels (67) Used to coat particles (68*) Structural molecule in polymeric matrices (68*)
Glucose-Oxidase Catalyzes oxidation of glucose to gluconic acid, produces H ₂ O ₂ as a byproduct	Glucose Oxidase-1 Glucose Oxidase-2	<ul style="list-style-type: none"> Available (69) High glucose specificity (70) Easily coupled to other molecules(71) (72) Stable(73) High turnover (70) 	<ul style="list-style-type: none"> Peroxide byproduct is toxic (74**) Active at low glucose concentrations (75) pH changes difficult to reverse rapidly (76) 	<ul style="list-style-type: none"> Coupled to insulin (75) Co-formulated with insulin glargine (77) Encapsulated in pH, hypoxia, and peroxide-sensitive polymer matrices (74**)
Boronic Acid Forms reversible esters with <i>cis</i> -diols	Boronic acid, Phenylboronic Acid, 3-Fluoro-4-Carboxy Phenylboronic Acid, Amino-Phenylboronic Acid	<ul style="list-style-type: none"> Small molecule Easily used as derivative (78) (60) Forms reversible, covalent bonds (79) (59) Glucose affinity in physiological range(17) PBA chemical properties can be modified using substituent groups (80, 81) (82) Sensitive to pH and glucose levels(83) No toxicity(84) 	<ul style="list-style-type: none"> Interaction not specific to glucose (85) Limited translational value Can degrade (17) 	<ul style="list-style-type: none"> Coupled to insulin for co-injection with polyols PBA polyol co-derivative insulin (17) Coupled to insulin via fatty-acyl linkers (86) Co-polymerized with diol-containing molecules in polymeric matrices (87)