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Smart Approaches to Glucose-Responsive Drug Delivery

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Abstract

A grand challenge in the field of “smart” drug delivery has been the quest to create formulations that can sense glucose and respond by delivering an appropriate dose of insulin. This approach, referred to as the “fully synthetic pancreas”, envisions *closed-loop* insulin therapy. The strategies for incorporating glucose sensing into formulations can be broadly categorized into three subsets: enzymatic sensing, natural glucose-binding proteins, and synthetic molecular recognition. Here, we highlight some examples of each of these approaches. The challenges remaining en route to the realization of closed-loop insulin therapy are substantial, and include improved response time, more authentic fidelity in glycemic control, improved biocompatibility for delivery materials, and assurance of both safety and efficacy. The ubiquitous existence of glucose, combined with the unstable and toxic properties of insulin, further compound efforts toward the generation of a fully synthetic pancreas. However, given the growing incidence of both type-1 and type-2 diabetes, there is significant potential impact from the realization of such an approach on improving therapeutic management of the disease.

1. Introduction

Diabetes, which is characterized by high fasting blood glucose levels (i.e. hyperglycemia), is increasing in prevalence globally.[1–3] Central to diabetes is the function of insulin, a small 51 amino acid signaling hormone produced by endocrine cells in the pancreas. Normally, insulin is secreted in response to elevated blood glucose levels and triggers cells in the body, specifically liver and skeletal muscle, to uptake and store glucose in its polymerized glycogen form for reserve energy.[4] There are a number of manifestations of diabetes that, though different in their mechanism, all result in a characteristic rise in fasting blood

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glucose. Type-1 diabetes, often called Juvenile Diabetes, has its onset primarily in children and is initiated by autoimmune destruction of insulin-producing beta cells located within pancreatic cell clusters known as the Islets of Langerhans, resulting in insulin deficiency.[5] Type-2 diabetes, meanwhile, primarily afflicts adults and is characterized by systemic insulin resistance in spite of normal and in most cases elevated insulin production by beta cells.[6] Gestational diabetes is transient insulin resistance occurring in pregnancy that is sometimes managed with insulin.[7] Additionally, diabetes and insulin deficiency can arise as a result of disease or infection (e.g. cancer or pancreatitis), genetic defects, β -cell toxicity from a medication or chemical, or a number of other known causes.[8]

Self-administration of exogenous insulin is an important component in managing diabetes. Type-1 diabetes is treated almost exclusively by exogenous insulin, while type-2 diabetes initially is addressed with a suite of small molecule pharmaceutical agents in combination with lifestyle changes.[6] However, advanced type-2 diabetes often progresses to insulin deficiency and necessitates an insulin regimen as well. For insulin-dependent patients, best therapeutic outcomes are observed when a strict insulin administration schedule is followed.[9] Though some patients respond well to insulin therapy, many suffer from complications that arise from poor adherence to therapy or from inadequate glycemic control.[10] Acute hypoglycemic episodes can result in coma or death.[11] Furthermore, chronic instability in blood glucose levels can lead to cardiovascular disease, non-healing wounds, and is an independent predictor of total, cardiovascular, and cancer mortality.[12] Though progress has been made in the generation of insulin variants with tunable pharmacokinetics,[13, 14] there remains a need to improve the fidelity of glycemic control to avoid both acute and long-term complications that arise in diabetes management.

One expanding area of research is the effort to develop “smart” or environmentally responsive delivery methods to improve therapeutic performance for drug delivery.[15] These strategies often rely on a trigger that can include physiologic indicators such as pH, the presence of proteins or enzymes, or disease-relevant analytes, in addition to strategies that have explored external triggers such as the application of heat, light, ultrasound, or a magnetic field, to localize therapeutic deployment.[16] The pursuit of “*smart*” strategies in the management of disease offers many potential benefits, including reducing the effective dose by increasing site-specific drug accumulation and limiting off-target effects, spatiotemporal control over therapeutic deployment, improved patient compliance, and control of therapy within the therapeutic window.

The development of “*smart*” drug delivery strategies for diabetes is particularly promising given the need to vary dosage based on real-time disease state of the patient. The ultimate goal for these efforts would be to create a “*Fully Synthetic Pancreas*”, an abiotic construct that can sense elevations in blood glucose and respond with a metered dose of insulin, and/or potentially glucagon, for closed-loop therapy.[17] However, recapitulating the natural dynamics of glycemic control, complete with both peaks and troughs, represents a major challenge in the design of a sensing material. The trigger for such a therapy, glucose, is a ubiquitous small-molecule analyte that is present in both healthy and diseased states, albeit in different concentrations. The insulin payload is a small protein with limited stability, prone to the formation of amyloid fibrils,[18] and can be lethal if dosed incorrectly. An ideal

system would respond quickly to elevation in blood glucose, but would promptly shut off to prevent insulin overdose. It should also not allow burst release, as that could lead to insulin overdose and hypoglycemia immediately following administration. The management of diabetes also necessitates life-long therapy. As such, the system must be amenable to facile serial administration by patients, have predictable and practical dosing schedules, be easily removable or have appropriate degradation and clearance kinetics, induce minimal inflammatory and fibrotic responses, be minimally immunogenic and provide predictable performance over time. These are among the many challenges that must be overcome in the development of a glucose-responsive insulin delivery system in order to realize the vision of a fully synthetic pancreas.

To date the most advanced approach towards closed loop therapy for diabetes is the development of automated control mechanisms based on a combination of digital pumps and glucose sensors.[19, 20] This approach has already shown promise in patients,[21] but currently requires the use of relatively bulky sensor and pump systems. An alternative approach has been to develop smart material-based systems or formulations. Early work established efforts for controlled release of insulin from polymeric materials.[17] Ethylene-vinyl acetate (EVAC) polymers were developed to provide glycemic control in diabetic rats for over 100 days.[22, 23] While efforts such as these could have utility for long-term basal insulin approaches, they do not afford the sensing and responsiveness envisioned by a fully synthetic pancreas. Efforts toward glucose-responsive insulin delivery have included a number of different materials and formulation routes, as described here. Glucose sensing in these systems has, in general, been provided by one of three different mechanisms: (i) enzyme-catalyzed pH changes, (ii) multivalent glucose-binding proteins, or (iii) molecular recognition by diol-binding chemical moieties. Here, we highlight these three general approaches.

2. Enzymatic Triggers

Enzymatic conversion-based actuation has been used to facilitate glucose responsiveness for insulin delivery. These methods have primarily relied on glucose oxidase (GOx), an enzyme that catalyzes the conversion of glucose into hydrogen peroxide and D-glucono- δ -lactone (which hydrolyzes to gluconic acid), frequently used in biosensors.[16, 24, 25] In the design of materials for glucose-responsive insulin delivery, the concomitant drop in pH through the conversion of glucose to gluconic acid can be used as a trigger for structural or conformational changes in materials. For example, some of the first examples of materials for glucose-responsive insulin delivery used polycationic membranes with embedded GOx, in which the membrane permeability changed as a function of glucose concentration.[26, 27] This principle was also extended to microcapsules prepared from amine-containing polymers.[28] The driving force for responsiveness in these systems arises from electrostatic repulsion of amine groups that become more protonated at acidic pH, leading to swelling of the membrane. In some instances catalase, a peroxidase enzyme intended to quench the hydrogen peroxide by product and convert it into an oxygen source for continued glucose oxidation by GOx, has been incorporated.[29–32] GOx has also been embedded along with catalase in microgels prepared from a naturally derived cationic polymer.[33] As glucose levels increased, these microgels swelled in diameter over 1.6 times, promoting accelerated

release of the encapsulated insulin. This approach was demonstrated to prolong normoglycemia in treated diabetic mice in comparison to insulin-loaded microgels without the addition of enzymes.

An alternative approach is to use materials that shrink (de-swell) in response to the drop in pH afforded by the conversion of glucose to gluconic acid. For example, studies have demonstrated the construction of a hydraulic flow controller from polyanionic hydrogels with embedded GOx,[34] as well as a membranous seal for a pressurized insulin reservoir prepared from poly(methacrylic acid).[35] In this case, the drop in pH as glucose is converted to gluconic acid causes the material to de-swell and collapse as repulsion is reduced among the side-chain carboxyl groups, increasing the pore size of the stoppering element to allow insulin release from a reservoir.[36] This collapsing hydrogel mechanism can also be applied for hydrogels loaded with insulin, whereby hydrogel de-swelling “squeezes” out encapsulated insulin.[37] Improving on the narrow pH range afforded by polyacids, others have explored more sensitive sulfonamide-based anions for more sensitive and responsive glucose-mediated properties.[38]

Another mechanism leveraging GOx sensing is the use of erodible or degradable materials prepared from acid-labile polymers, such as polyesters, that can degrade more rapidly in the presence of elevated glucose levels, leading to enhanced release of encapsulated insulin.[39] Building on their early efforts with EVAC, GOx has been embedded into materials to accelerate degradation in response to glucose levels.[40] Microgels prepared through cross-linking by a pH sensitive linker were demonstrated to swell and release insulin as cross-linking groups were cleaved.[41] Another effort encapsulated GOx within nanoparticles of acetyl-modified dextran, an acid-degradable polymer.[42] These nanoparticles were coated in either alginate or chitosan, and when the two particle types were mixed, an injectable shear-thinning nanogel was formed. The material demonstrated glucose-mediated release of insulin from particles, and was able to restore normoglycemia in diabetic mice for up to 10 days. Polymeric vesicles from amphiphilic polymers with encapsulated insulin, GOx, and catalase have also been explored.[43] The hydrophobic block, consisting of ketal-modified poly(serine), becomes more hydrophilic when the acid-labile ketal group is cleaved, causing vesicle rupture. These vesicles demonstrated glucose-responsive insulin release and maintained normoglycemia in a diabetic mouse for up to 5 days.

3. Glucose-Binding Proteins

Glucose-responsive materials have also been prepared using lectins, a family of carbohydrate-binding proteins, as natural receptor-based glucose-sensing elements. The most frequently evaluated of these lectins is concanavalin A (ConA), a tetravalent binding protein.[44] Some of the first work in this area was reported in 1979 with the demonstration of glucose-responsive release of a glycosylated insulin derivative from an insulin-ConA complex.[45] Variations on this approach were explored in the years that followed through immobilization of ConA onto polymers or incorporation within microcapsules for controlled release of glycosylated insulin.[46–50] Glycosylation of insulin, while preserving the bioactivity of the protein, enables reversible binding to ConA and glucose competes with the

glycosylation moiety for binding to ConA; therefore a rise in local glucose concentration drives equilibrium toward the liberated modified insulin protein.[51]

Another approach leveraging the binding specificity of ConA for carbohydrates has used the protein as a tetravalent cross-linker in the preparation of polymeric hydrogels. Both naturally occurring polysaccharides and synthetic polymers containing saccharide-like substituents have been explored in this approach.[52–55] The glucose-responsive swelling exhibited by these materials as free glucose disrupts their ConA-mediated cross-linking could be used to prepare hydrogel devices with encapsulated insulin that release this payload as they swell in response to glucose. Specifically, the porosity of polymers cross-linked by both covalent and ConA-saccharide methods can be used to produce materials with both stable and transient cross-links, the ratio of which can be altered to modulate the swelling properties of the material in response to glucose.[56] This method of mixed cross-linking was used to demonstrate precise controlled release of a model protein, lysozyme, once glucose was added to the hydrogel.[57] Glucose moieties appended to a polymer can also be used, in combination with ConA, to prepare materials that undergo a *sol-gel* transition in response to glucose levels.[58, 59] These *sol-gel* materials were incorporated into a diffusion cell device with a poly(hydroxyethyl-methacrylate) membrane, the porosity of which allowed the diffusion of glucose and insulin, but not ConA or the glucose-modified polymer.[60] The device demonstrated glucose concentration-dependent release of proteins from the chamber as the *sol-gel* transition occurred. Through similar principles, ConA gating was used to cap the pores of carbohydrate-modified mesoporous silica, enabling glucose-responsive release from particles.[61] ConA-based approaches may suffer from possible toxicity associated with this non-human protein, and strategies have explored covalent attachment of ConA to a material to limit its diffusion.[62–64]

4. Synthetic Molecular Recognition of Glucose

Instead of using natural glucose-sensing elements such as lectins or enzymes, some have explored the use of synthetic molecules that can bind to glucose. Pioneering work first demonstrated the use of phenylboronic acids (PBAs) to bind to glucose and other similar diols.[65] PBAs are Lewis acids known to bind reversibly to *cis*-1,2 or *cis*-1,3 diols, such as glucose, which stabilizes a negative charge on the boronic acid.[66] This binding group has also been used to prepare glucose-responsive hydrogels.[67–70] In these studies, a copolymer bearing a pendant PBA group is combined with poly(vinyl alcohol) (PVA), a polyol. The mechanism for glucose-responsiveness in this type of system arises from a disruption in cross-linking as free glucose competes with PVA for binding to the PBA-containing polymer. A consideration in the creation of these PBA-containing polymers is the pKa of the boronic acid, as PBA only binds to glucose at pH at or above its pKa.[71] The pKa of many early polymers was around 8.6, and therefore these strategies were not functional at physiological pH.[72] The incorporation of amine-containing groups in these PBA polymers serves to reduce the effective pKa of neighboring boronic acids, enabling these systems to function at physiologic pH.[72] Another mechanism by which PBA-containing hydrogels could respond to glucose is through charge-charge repulsion-induced swelling. PBA bound to glucose is a more stable species than the neutral PBA unbound to glucose.[29] Therefore, if the pKa of the PBA is appropriately tuned, an increase in free

glucose will drive equilibrium toward the more stable charged species. Repulsion of these charges can lead to swelling of hydrogels, which has been especially evident when combined in coordination with monomers that exhibit thermally-responsive changes in solubility, a so-called lower critical solution temperature (LCST).[67] PBA inclusion lowers the LCST of these polymers, but upon glucose addition the LCST increases promoting a phase-change in physiologically relevant conditions. PBA may also be used for glucose-responsive self-assembly of PBA-modified peptides in order to deliver insulin from within peptide hydrogels.[73]

The use of PBAs has extended into the area of chemically modified insulins as well. PBA-containing polymers immobilized within a material have been used for controlled release of gluconated insulin,[72] leveraging interaction of the PBA-glucose in a way similar to that described above for ConA-glucose. An alternate approach has evaluated the direct covalent modification of insulin with PBA groups to prepare glucose-responsive insulins.[74–76] In combination with glucamine-containing polymers, PBA modified insulins were demonstrated to undergo glucose-responsive release.[76] Alternatively, insulin modified with both PBA and a glucose-like diol were demonstrated to form aggregates that dissociate in the presence of elevated glucose levels.[75] Recently, site-specific modification of insulin with aliphatic PBA-conjugates at the B-chain lysine was investigated.[74] In these studies, improved glucose control was demonstrated for the PBA-modified insulin in comparison to native and standard long-lasting insulin.

5. Future Directions

Here we described three basic approaches to preparing glucose-responsive materials for drug delivery, with sensing strategies that leverage both native glucose recognition as well as synthetic molecular recognition. The materials prepared from these approaches have most frequently been hydrogels, which have properties that facilitate diffusion of glucose for more rapid sensing and equilibration, as well as tunable mesh size for selective release of insulin in response to glucose. The materials described undergo a variety of changes, with demonstration of materials that swell, shrink, or degrade in response to glucose. Each approach has its own drawbacks, from possible immunogenicity of the protein components in the case of GOx or ConA, to poor glucose selectivity and often limited function in physiologic conditions for PBA-based systems. In addition, the biocompatibility of the delivery materials and their feasibility for serial patient-administered use must be addressed. Treatment of diabetes must continue for a lifetime, and this imposes additional considerations when implementing strategies evaluated thus far. Future directions should be taken to address some of these issues. Strategies that have proposed functionalization of the insulin protein itself, either through PBA or through glucose-like groups in combination with glucose-binding materials, may offer some possible benefit in shortening the response time to elevated glucose, especially when compared to the often slow kinetics for material transition or degradation. These strategies may also circumvent the need for a drug delivery material, and the sensing element can be directly fused to insulin. However, modification of the insulin protein may affect immunogenicity, and would require thorough investigation as a new molecular entity in order to achieve approval. A glucose-sensing material or excipient in combination with authentic native insulin, alternatively, may reduce some of these

regulatory barriers. Demonstration of both safety and efficacy in preclinical and early-stage clinical studies, along with predictable function over long periods of time, remains a challenging hurdle in order to realize the vision of glucose-responsive insulin therapy.

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