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Abstract

This review is focused on the materials and methods used to fabricate closed-loop systems for type 1 diabetes therapy. Herein, we give a brief overview of current methods used for patient care and discuss two types of possible treatments and the materials used for these therapies–(i) *artificial pancreases*, comprised of insulin producing cells embedded in a polymeric biomaterial, and (ii) totally *synthetic pancreases* formulated by integrating continuous glucose monitors with controlled insulin release through degradable polymers and glucose-responsive polymer systems. Both the artificial and the completely synthetic pancreas have two major design requirements: the device must be both biocompatible and be permeable to small molecules and proteins, such as insulin. Several polymers and fabrication methods of artificial pancreases are discussed: microencapsulation, conformal coatings, and planar sheets. We also review the two components of a completely synthetic pancreas. Several types of glucose sensing systems (including materials used for electrochemical, optical, and chemical sensing platforms) are discussed, in addition to various polymer-based release systems (including ethylene-vinyl acetate, polyanhydrides, and phenylboronic acid containing hydrogels).

Keywords

artificial pancreases; islet encapsulations; alginate hydrogels; glucose responsive polymers; electrochemical and optical glucose sensors

1. Introduction

Type 1 diabetes is an autoimmune disease that results in the destruction of insulin producing beta cells in the islets of Langerhans.[1] According to the American Diabetes Association, type 1 and type 2 diabetes affects over 25.8 million people in the United States or about 8.3% of the population.[2] Furthermore, the direct and indirect costs of diabetes totaled $174 billion in 2007 in the USA.[2] This review discusses the materials used in the devices and implants used to treat diabetes. Current treatments often involve daily injection of insulin from recombinant human or animal sources.

The regulation of insulin secreted by the beta cells of the pancreatic islets in response to blood glucose is a highly dynamic process. Briefly, the endocrine cells of the pancreas are grouped in the islets of Langerhans. Each islet has a population of alpha cells, which secrete glucagon, and a population of beta cells, which secrete insulin.[3] The concentration of glucose in the blood is regulated by insulin and glucagon, which are antagonistic hormones. Insulin lowers blood glucose levels by stimulating virtually all body cells except those of the brain to take up glucose from the blood. Blood glucose is also decreased by insulin through slowing the liver's breakdown of glycogen, a polymeric form of glucose, and inhibiting the conversion of amino acids and fatty acids to sugar. Glucagon regulates blood sugar levels by signaling liver cells to increase glycogen hydrolysis, convert amino acids and fatty acids to glucose, and start releasing glucose back into circulation. Insulin is made when proinsulin is released from the pancreas and split into insulin and C-peptide in a one to one ratio.[3] C-peptide repairs the muscular layer of arteries and has beneficial therapeutic effects on many complications from diabetes.[4, 5] Another crucial component of blood glucose level regulation is somatostatin, which regulates the endocrine system through inhibition of both insulin and glucagon release.[3]

Autoimmune destruction of beta cells in the pancreas leads to type 1 diabetes and an insulin deficiency. The ensuing lack of insulin results in increased glucose levels, which can be fatal

unless treated. Currently, type 1 diabetics depend on daily injections of insulin or insulin pumps combined with glucose monitoring through meters. Complications arising from both high and low blood sugar levels are problematic for diabetics, in both acute and chronic timescales. High blood sugar levels cause fatigue and damage to organs and joints. Hypoglycemia can induce seizures, unconsciousness, brain damage, or death and is especially dangerous for diabetics while they are asleep.[3] Herein, the current state of developing therapies and the materials used in those therapies are discussed.

2. Introduction of Therapeutics: Closed vs. Open

2.1 Open-loop systems

In general, open-loop insulin delivery systems combine external insulin pumps with continuous glucose monitoring via a subcutaneous sensor. These systems are externally regulated through a variety of triggers, and ultimately rely upon patients to activate the system. For example, magnetically triggered systems are comprised of drug molecules and magnets or magnetic particles dispersed in a solid polymeric matrix. In the presence of an external oscillating magnetic field, the drug is released.[6] Both non-degradable, diffusion controlled, and degradable, erosion-controlled polymers have been developed to release drugs when exposed to ultrasound.[7, 8] Another example of open-loop systems is electrically controlled systems. In these systems, an applied electrical field can modulate drug flux.[9]

The basic elements of diabetes treatment involve accurate glucose sensing coupled with appropriate insulin dosing. There are a variety of ways to achieve these two components, however. In current protocols for treating diabetes, sensing and delivery are wholly separated (“open-loop”). Testing informs dosing, but only via patient or physician interaction. There have been efforts towards algorithms that can accurately translate the same information, allowing for sensors to be directly coupled to a delivery vehicle (“closed-loop”), but thus far they have proved inefficacious in achieving normoglycemia.[10]

With the advent of electrochemical glucose sensing,[11] patients have better tools to measure their blood-glucose levels. Current monitoring systems, for the most part, are based on the measurement of a sample of patient blood. They use disposable test strips that amperometrically determine the concentration with the help of an enzyme, typically glucose oxidase or glucose dehydrogenase.[11] The blood sample is most often taken by a finger-prick or a forearm-prick, and then as little as 600 nL[12] of blood is loaded into the testing well of the strip, which is connected to a device that applies a voltage and measures the calibrated current response, yielding a value for blood-glucose concentration. These readings offer the patient an up-to-the-minute analysis of their blood-glucose levels. Systems for this sort of monitoring are sold by many companies, including Johnson & Johnson (LifeScan, One Touch Ultra), Arkray (Glucocard X-Meter), Bayer (Ascensia Contour), Abbott (Free-Style, Precision Xtra), and Accu-Chek (Aviva).[13-17] Insulin dosing amounts are calculated from the information derived by blood glucose measurements.[18, 19] Although blood draws are still the most prevalent method of monitoring, there are several systems that have come to market designed to be long-term, implantable glucose sensors, such as SCGM1 from Roche Diagnostics and GlucoDay marketed by A. Menarini IFR.[20, 21] These subcutaneous systems are currently functional for up to one week, after which time they must be replaced owing to protein deposition and a reduction in amperometric response.

Insulin is still frequently delivered via subcutaneous injections. Solutions to the frequency of injections has come in the form of long-acting and short-acting formulations of insulin, which are available commercially through Eli Lilly and Lantus.[22] An injection is self-

administered several times daily at meal-times, to handle the bolus (short-term) and basal (long-term) doses. These injections attempt to replicate the insulin normally delivered by a healthy pancreas. In an effort to alleviate the quantity of painful and repeated injections, there has been movement towards using insulin pumps.[1, 23] A pager-sized device connected to a catheter subcutaneously implanted in the patient externally controls the pumps. These devices are aimed at providing a constant basal dose of insulin, but are capable of delivering boluses at meal-times. The catheters are replaced intermittently, however, the level of comfort and control is increased with these systems.

An alternative approach is provided by Medtronic, which offers the MiniMed MMT-2007D implantable insulin pump (available for patients in Europe).[24] This pump is unique in that it is implanted in the peritoneal cavity of the patient (the intraperitoneal or i.p. space). This has tremendous advantages over subcutaneous delivery in that blood glucose stability is significantly improved via i.p. delivery.[25-27] It is comprised of an insulin reservoir, which can be refilled with fresh insulin every 6-8 weeks via a tube that runs up to the subcutaneous area of the abdomen. It delivers insulin directly into the i.p. space via a specially designed catheter. Such a pump is wholly implanted and lasts for 7-10 years inside the patient.[28]

Combinations of these sensing and delivery mechanisms have yet to achieve a perfect balance for patients.[10] This review covers some of the current systems and their materials science aspects, as well as focuses on new and developing technologies and the materials they are comprised of that offer cutting edge approaches to diabetes therapeutics.

2.2. Closed-loop system

In a closed-loop insulin delivery system, real-time data of glucose concentration is provided to an insulin source, which is able to respond with the precise amount of insulin necessary to maintain correct control of blood glucose levels. “Closing the loop” has often been termed the “Holy Grail” of type 1 diabetes therapy, since continuous glucose monitoring is intimately linked with an insulin pump.[29, 30] Two types of closed-loop systems are described: explicit and implicit.

2.2.1. Explicit closed-loop—An explicit closed-loop system is one in which an insulin pump is interfaced with a continuous glucose monitor (CGM) and controlled through an insulin infusion algorithm.[29] These systems rely on the ability of the algorithm to accurately dispense the correct amount of insulin to maintain normoglycemia. The CGM sensor is typically implanted subcutaneously; therefore, key challenges in this area lies in the discrepancy between glucose levels in subcutaneous tissue and blood[31] and preventing biofouling of the sensor.[32] Poor biocompatibility limits device lifetime.[32]

2.2.2. Implicit closed-loop systems—Implicit systems are those in which the device, be it a polymer-based drug delivery system or transplanted cells, senses glucose and responds by eluting insulin, or other important hormones, peptides, or small molecules, in direct response to glucose levels.[31] This review examines two types of implicit systems: (1) the artificial pancreas in which insulin producing cells are encapsulated in a material and transplanted into a recipient and (2) a synthetic pancreas which contains a sensing component and a releasing component, which may be combined into a “smart” insulin-releasing polymer.

3. Artificial Pancreas: Encapsulating Cells

Transplants of exogenous beta cells have been performed experimentally in both mice and humans, with significant proof-of-principal data being generated. However, allogenic cells typically invoke a foreign body response requiring long-term immunosuppressive drugs to

protect the transplanted tissue.[33] An alternative technique is to encapsulate transplanted beta cells are in a semi-permeable container, isolating and protecting them from the immune system (Figure 1(a)).[34, 35]

Isolating the encapsulated islet cells from the host immune system must not inhibit the transport of small molecules and proteins, such as glucose, oxygen, and insulin. Materials used in this process must also be biocompatible, since materials themselves can induce a host response.[35] Implantation of biomaterials initiates a healing response by monocytes and neutrophils. Propagation of fibroblasts and vascular endothelial cells follows. As early as 3 to 5 days following implantation, granulation tissue can form. Ultimately, granulation tissue will form a fibrous capsule surrounding the implant.[36] A material that achieves immuno-isolation but obstructs mass transport of nutrients will result in cell death. Furthermore, materials that allow diffusion of nutrients but maintain a molecular weight cut off that prevents insulin and other hormones from freely diffusing will be inappropriate for therapeutic use. Just as important as diffusion, the biocompatibility of the material can result in device failure through host protein adsorption and fibrous encapsulation, which results in preventing insulin from diffusing out of the polymeric container and into the recipient.[37]

This section will discuss materials and methodologies for immune-isolating islets for transplantations including: islet encapsulation, conformal coatings, and planar sheets.

3.1. Islet encapsulation

Microencapsulation provides a simple way to enclose bioactive materials within a semi-permeable polymeric membrane for the purpose of protecting the bioactive materials and releasing the enclosed substances or their products in a controlled fashion. One such method of encapsulation is through electrostatic droplet generation. For electrostatic droplet generation, a high voltage generates a temporary electrostatic charge on the beads during formation. This charge optimizes the spherical shape of the beads and eliminates bead clumps and coalescence. For polyanionic materials such as alginate, beads can be crosslinked with a divalent cation, such as barium chloride or calcium chloride, to form a gel.[38] Various materials have been used as biopolymeric coatings in islet microencapsulation. These have included alginate,[39] agarose,[40, 41] tissue-engineered chondrocytes,[42, 43] polyacrylates,[41, 43] and poly(ethylene glycol) (PEG)[44, 45]. Alginate-poly-L-lysine (PLL) -based microencapsulation of islets, first described by Lim and Sun,[39] were reported not to interfere with cellular function, and these microcapsules have been shown to be stable for years in small and large animals.[46-51] Additionally, several materials have been incorporated into alginate to reduce plasma adsorption, as semi-permeable membranes, and to alter the mechanical properties of the encapsulation material, such as PEG[45] and PLL.[52]

Another method for microencapsulation recently described by Dang et al.[53] employs micromolding systems based on polypropylene meshes. The polymer meshes were immersed in alginate solutions with and without INS-1 cells (a rat insulinoma cell line). Immediately after, the mesh was immersed in a crosslinking solution of calcium chloride. To release the particles, the mesh was agitated in buffer. This process also holds the ability to exclusively coat one side of the particles with a polycation, such as PLL, which may have advantageous properties in directed assembly of injected materials. This technique is an inexpensive method for encapsulating cells and is also capable of producing shapes not accessible through electrostatic droplet generation, such as squares, by changing the shape of the mesh pore. The viability of these cells along with their insulin response properties were maintained through this encapsulation process.[53]

Soft-lithography techniques for production of alginate particles have been developed by Qui et al.[54] Through the use of polydimethylsiloxane (PDMS) templates, Qui et al.[54] were able to control the size of the particles very precisely. To formulate particles, a PDMS mold was fabricated using a 10:1 mixture of PDMS and curing agent, which was poured onto a silicon master. The silicon master was lithographically patterned to produce the size and shape of particles desired. An alginate solution was poured into the PDMS mold and was crosslinked using a divalent cation solution, such as barium chloride or calcium chloride. The alginate gelled and were subsequently released in buffer.

3.2. Conformal coatings

Traditional electrostatic extrusion of islets encapsulated in alginate in coaxial gas flow represents one method to fabricate capsules. However, the position of encapsulated islets and thickness of the coating are difficult to control with this approach. It is estimated that during transplantation, 96% of the transplanted volume consists of alginate rather than islets, meaning that a therapeutic dose of 10 mL of islet tissue requires implanting 270 mL of microcapsules.[55] Repeated transplantations only serve to exacerbate this problem. Furthermore, islets commonly protrude from capsules formulated through electrostatic encapsulation, which elicits foreign body responses post-transplantation.

An alternative method to electrostatic microcapsule encapsulation, namely the conformal coatings of islets, presents as a solution to both the dosing volume and foreign body response problems. A wide variety of chemical functional groups can be conjugated to, or bioactive substances immobilized on the surface of, a cell via covalent conjugation, hydrophobic interaction, or electrostatic interaction.[56] Methods for conformal coatings are illustrated in Figure 1(b). Covalent conjugation of polymers to living cells has typically been achieved through reactions between the amino groups of membrane proteins and *N*-hydroxyl-succinimidyl ester (NHS) or cyannuric chloride.[56] Islets chemically modified with a poly(ethylene glycol) (PEG) layer, which prevent foreign body responses thus abating the immunogenicity of the implanted islets, serve as effective therapy in diabetic rodents for at least 1 year when accompanied by cyclosporine, a commonly used immunosuppressant.[57] Unmodified islets remained viable for only 5 days without immunosuppressive therapy and 12 days with therapy.[57] Another study of PEG modified islets demonstrated that a systemic over expression of heme oxygenase-1 (HO-1), a powerful cytoprotective agent that excels in inhibiting non-specific inflammation during the early stages following islet transplantation, results in complete survival of transplanted islets for 100 days without islet function impairment, when employed with a low dose of cyclosporine (1 mg/kg/day).[58] Complete rejection of islets, even with co-treatment of HO-1 expression and cyclosporine, occurred within 2 weeks.[58]

A blood-mediated inflammatory reaction is triggered by islets when they come into contact with blood in the portal vein, resulting in the production of monocyte chemoattractant protein-1, a cytokine that recruits macrophages.[59] Islet surface modification via covalent attachment of heparin to reduce this blood-mediated inflammatory reaction in transplantations has also been studied.[59] Heparinizing islets as opposed to systemic delivery of heparin presents an attractive alternative over systemic delivery in preventing the blood-mediated inflammation response to implants. Islets were biotinylated through exposure to an NHS conjugated biotin. The resulting islets were incubated with avidin and heparinized through macromolecular conjugates of heparin binding with the biotin/avidin complex. The resulting modified islets retained similar insulin release properties compared to unmodified islets. Allotransplantations of heparinized and unmodified rat islets from the same donor resulted in normoglycemia for 30 days.[59] In porcine models, allotransplantations of unmodified islets resulted in clotting and an increase in thrombin/

antithrombin, an indicator of instant blood-mediated inflammatory reaction. Allotransplants of heparinized islets attenuated thrombin/antithrombin levels and thrombi were scarce in the portal branches of the recipients.[59]

Hydrophobic interactions between the hydrophobic alkyl chains of amphiphilic polymers and lipid bilayer membranes provide another method for conformal coating of islets. Poly(vinyl alcohol) (PVA)-PEG-lipid conjugates were spontaneously incorporated into cell membranes through hydrophobic interactions between the lipid bilayer of the cell membrane and the lipid portion of the conjugate.[40] Upon glucose stimulation, the PVA encapsulated islets responded similarly to unmodified islets. Enzymes, such as urokinase, can also be immobilized on islet cell surfaces through hydrophobic interactions with PVA derivatives.[60] The islets modified with urokinase displayed fibrinolytic properties, suggesting blood coagulation could be controlled. No significant differences in insulin response to glucose challenge assays were observed between the urokinase modified islets and control islets.[60]

Electrostatic interactions between negatively charged cell surfaces and cationic polymers are another possibility for coating islets. Further modification via layer-by-layer addition of anionic and cationic polymers allows for precise control of conformal coating thickness. Conformal coatings were first demonstrated by Zekorn et al.[61] in which a discontinuous density centrifugation gradient with the top most layer composed of islets suspended in sodium alginate was used to encapsulate islets. Denser spacer layers of ficoll contained barium chloride. During centrifugation, islets coated in sodium alginate cross the layer containing barium chloride and the alginate is cross-linked. Perfusion and static incubation glucose challenges of conformally coated islets exhibited the same insulin release properties as control islets. Calafiore et al.[62] reported that conformal coatings of alginate on islets immunoprotected both allo- and xenogenic transplants in diabetic rodent recipients. Upon transplantation in higher order mammals, conformal coatings were reported to fully immunoprotect islet allographs and temporarily protected xenographs.[62]

3.3. Planar Sheets

Planar islet sheets employ a reinforcing mesh, which adds mechanical strength and durability to the system, seen in Figure 1(c). Typically, an acellular alginate layer uniformly coats the sheet to form an immunoprotective barrier. Much like microencapsulated systems, the alginate layer serves to prevent host rejection of encapsulated cells while permitting diffusion of insulin, nutrients, and waste products.[63]

Colton[64] has reported that the oxygen available to immunoisolated islets is reduced by 50% when crossing a barrier 25μm thick. Unlike microencapsulated islets, the immunoprotecting layer in planar sheets can be as thin as 50-75μm.[63] The 200μm barrier for the microencapsulated particles was reported to reduce oxygen flux and potentially impair insulin production. Another potential advantage of planar sheets is the high tissue density loading that is possible. Islet sheets are capable of having 50% (v/v) islets, which reduces the necessary volume for transplantation, although possibly increasing oxygen requirements.[63]

In addition to alginate, PVA hydrogels have been used to form islet sheets.[65] Xenotransplantation of rat islets encapsulated in PVA hydrogel sheets to diabetic mice resulted in significantly lower glucose levels for 30 days.

4. Completely Synthetic Pancreas

4.1. Sensing—Continuous Glucose Monitoring (CGM)

4.1.1. Smart Materials for Pancreas Replacement—As discussed previously, closed-loop delivery may be subdivided into two classes: explicit and implicit. Explicit methods involve a glucose sensor coupled to an insulin and glucagon delivery system via an algorithm for controlling dosing. These algorithms attempt to regulate blood glucose levels through releasing insulin based upon sensor readings.[66] Human studies indicate that these approaches can significantly improve glucose control. While promising, challenges remain, and algorithms in combination with existing sensor and pump technology, have not yet been able to induce normoglycemia. Implicit methods involve completely self-regulating materials that sense and deliver insulin independent of computational, patient, or physician intervention.

Totally synthetic pancreases are based on chemically-derived materials that will respond to glucose concentration by releasing insulin in a complimentary and controlled way, circumventing the need for explicit methods. This section will focus on the two major facets of the closed-loop approach. The first aspect is long-term, continuous glucose sensors, which encompasses materials that have been developed for glucose detection and monitoring, including nanotube based materials and electrochemical sensors. The second is glucose-responsive polymer materials, such as hydrogels, and degradable materials. These materials form the basis for the synthetic pancreas.

4.1.2. Electrochemical Detection of Glucose—Most attempts at explicit closed-loop systems involve an implanted electrochemical glucose sensor.[24, 67] These sensors must allow for rapid and accurate glucose measurement. Electrochemical glucose sensing, particularly using blood or peritoneal fluid, is perhaps the best available method for accomplishing these requirements. Blood-glucose test strips, for example, also rely on the electrochemical detection of glucose. Such sensors, whether implantable or external, typically consist of an enzyme, glucose oxidase (GOx) or glucose dehydrogenase (GDH), which is coupled to an electrochemical cell. A comprehensive review of the GOx enzyme was written by Wilson & Turner[68] in which GOx is discussed in detail. The enzyme is typically immobilized in a polymer matrix near the electrode surface, thus allowing for rapid detection via small-molecule mediators of the electrochemical processes. Heller & Feldman have made significant advances in blood-glucose monitoring and home test strips for diabetic patients, and have written several reviews on this topic.[69, 70] Enzymatic detection is a reliable and sensitive technique and still represents the most accurate sensing method. The reaction being monitored is the oxidation of glucose to gluconolactone, which rapidly hydrolyzes to gluconic acid. This is accomplished by the GOx enzyme, containing two molecules of its cofactor, flavin adenine dinucleotide (FAD) (Figure 2-c). This cofactor undergoes reduction as glucose is oxidized. It is also possible to detect the oxidation of glucose directly, via non-enzymatic methods.[71] These chemistries are used in external test strips, wire-based intravenous and subcutaneous sensors, and other implantable monitoring systems.[69]

Materials for Mediator-less Glucose Detection: Enzymatic sensors face several challenges as components of closed-loop systems. One of these complications arises from the insufficient stability of the enzyme that arises from the ability of non-native environments to degrade the enzyme.[71] This is especially important during the fabrication process, at which point the enzyme may be exposed to harmful environments. In particular, pHs below 2.0 and above 8.0 have shown significant decreases the enzymatic activity of GOx.[72, 73] Elevated temperatures – above 40°C – also result in degradation.[74] Another fabrication issue making

non-enzymatic detection a more attractive option, is the immobilization of the enzyme.[71] The reproducibility of assembling these devices is crucial in maintaining consistent activity of the enzymes. For these reasons, non-enzymatic glucose detection is of great interest in designing an artificial pancreas.

Efforts towards overcoming the fabrication complications inherent in mediator based enzymatic sensors immobilized in polymers include the use of specially designed electrode materials, which are often fabricated with nanostructured metals, metal-oxides or alloys.[75, 76] Willner et al.[77] directly conjugated FAD to a gold electrode. They achieved this via immobilization of an organic disulfide monolayer – cystamine –on a gold surface through the interaction of the thiol group with the gold. Pyrroloquinoline quinone (PQQ) was covalently linked to the monolayer through a condensation reaction. The carboxylic acid group on the PQQ was activated through carbodiimide chemistry, allowing for modification of the surface with FAD. Subsequently, the fully functional form of GOx was reconstituted onto the Au-FAD surface through treatment with apo-GOx, which is missing the FAD co-factor. This resulted in a fully integrated electrical contact between the gold electrode surface and the enzyme, allowing for a more direct variant of enzymatic-based electrochemical sensing, one without the typical need for a mediator and for a polymeric immobilization agent for the enzyme in question.[77] These systems still suffer the fabrication limitations inherent in employing enzymes. To alleviate these restrictions, alloy systems devoid of enzymes were prepared through combinatorial methods by Mallouk et al.[78] The most successful alloys were able to oxidize glucose at substantially more negative potentials than pure platinum. Poisoning agents – such as ascorbic acid, uric acid, and 4-acetamidophenol – were oxidized at more positive potentials, thus enhancing the glucose signal and specificity.[78]

Other attempts at non-enzymatic sensing include the use of conducting polymers, such as poly(aniline) (PANi), to mediate glucose oxidation. Phenylboronic acid (PBA) units are known to bind to sugars, specifically to 1,3-diols such as glucose[78, 79][75, 76]. Such a system was developed by Freund & Shoji, who copolymerized aniline with a boronic acid modified aniline monomer.[79] A glassy carbon electrode was coated with the polymer, referred to as poly(aniline boronic acid), and then subjected to varying biologically relevant glucose concentrations in vitro at physiological pH. The oxidation of glucose to gluconic acid resulted in a local pH change, which affected polymer conductivity. The PBA moiety brought the sugar in close proximity with the electrode. A linear correlation between the measurable change in current from oxidation and the concentration of glucose was found in the 3-14 mM range (54-252 mg/dL). One pitfall of this approach is that the poly(aniline boronic acid) is not specific for glucose. Interfering sugars – such as fructose – have a higher affinity for the enzyme binding site, resulting in much higher signals for non-glucose molecules. This results in a nonspecific signal for blood glucose levels and may lead to false readings.[79-81] This drawback does not exist for GOx-based sensors, as these enzymes are inherently glucose-specific in their binding event. Future work includes the development of polymer systems with bis-boronic acid moieties, used in other cases[78] for enhancing the specificity of glucose-binding, while reducing the interference by other sugar moieties.[78]

Materials for Redox-mediation of GOx: Electrochemical glucose sensing requires coupling the active enzyme to the electrode such that the accompanying potential of the reduction of FAD can be monitored. This has been accomplished with a variety of mediators (organic, inorganic, and hybrid materials).[82-86] Another byproduct of glucose oxidation is peroxide, which can be detected by the use of horseradish peroxidase (HRP).[82, 84-86] Inorganic mediators aid in detection of the oxygen/peroxide elements of the GOx reaction. They are often not ideal due to solubility issues associated with O_2, however there are commercial sensors available which use these elements, along with $Fe(CN)^{-3/-4}$ mediators.

Organic/Inorganic hybrid redox mediators are often a polymer with a pendant redox center. Heller et al. used GOx immobilized inside materials, such as hydrogels, bearing electroactive redox centers based upon osmium complexes. Notably, an electron-conducting hydrogel system was constructed using poly(4-vinylpyridine) with a tethered $Os^{+2/+3}$ complex with three di-*N*-alkylated-2,2'-diimidazole units.[83, 84] Figure 2-a shows the chemical structure of such a hybrid mediator system. The polymer was water soluble and was combined with GOx. Such materials can be subsequently crosslinked into a hydrogel using a variety of materials, such as polyethylene glycol diglycidyl ether, followed by a hydration step.[85] The tether was 13 atoms long and the pyridine units on the backbone were quaternized at the tether site, for water solubility. These redox centers effectively shuttle electrons to the electrode during the oxidation of glucose by GOx. Another system by Willner et al. employed Au nanoparticle composites with oligoaniline moieties for GOx mediation.[86] The result was a sensitive and glucose-selective bioelectrocatalytic electrode system with the potential for miniaturization.

Organic redox mediator materials have historically been small molecule systems, although they also include polymeric quinones.[87] Polymers are more ideal than small molecule mediators, as they cannot leech from the electrode surface and cause errors or drifts in the observed current. Recently, conjugated polymers have found utility as mediators. Thompson et al. have accomplished the direct-wiring of GOx to a conducting polymer.[88] They employed vapor phase polymerization (VPP) of poly(3,4-ethylenedioxythiophene) (PEDOT) followed by incubation with a solution of GOx. The resulting composite film exhibited clear peaks in the cyclic voltammogram corresponding to the redox behavior of GOx at -0.58V and -0.43V (vs. Ag/AgCl).

Implantable Sensors for Continuous Glucose Monitoring Systems (CGMS): Continuous monitoring of glucose is an essential component of a closed-loop delivery system. Many electrochemical sensors have been designed for implantation in the body, and considerations have been made for anti-biofouling. The sensors can often remain accurate for up to one week, and commercially available CGMS monitors, such as the DexCom SEVEN+, the Medtronic MiniMed, and the Abbott FreeStyle Navigator, are user-replaced. [13, 28, 89] Thus far, there has been no more permanent solution to electrode fouling, which leads to significant reading errors.[90] Promising work in implantable sensors includes the development of user-replaced subcutaneous systems that are commercially available.[91] Much work has been done on membranes, often comprised of derivatized poly(urethanes) that serve to enhance anti-biofouling and prolong sensor lifetimes. Titanium is often used as a protective housing for implantable devices, to prevent fibrosis over the active portion of the implant. Further, materials such as hydrogels, phosphorylcholines, poly(urethane)s derivatized with polar phospholipid groups, and innumerable others are also used for the organic interfaces of the implant, which are required for both sensing and delivery components of the devices.[90] These materials must be mechanically robust, as well as biocompatible.[92] Several implanted systems are based on microdialysis, available as SCGM1 from Roche Diagnostics[20] and GlucoDay from A. Menarini IFR.[21]

Some sensors are based on the detection of oxygen, such as the implantable subcutaneous sensor developed by Lucisano et al.[93] It comprised GOx immobilized in a protein hydrogel matrix of albumin and glutaraldehyde. The sensor arrays were fabricated on an alumina disc and were covered by a layer of PDMS that had wells over each platinum electrode, which contained the GOx/hydrogel composite. The housing for the system was titanium; the device was hermetically sealed. Figure 2-b shows an image of this device. The sensor was implanted subcutaneously in pigs and the blood glucose signals were calibrated after two weeks. This time scale was chosen due to the initial signal loss reaching an asymptotic value approximately two weeks after implantation. From then on, monitoring proceeded for close

to two years and the glucose concentrations were correlated with blood-glucose levels. As with all subcutaneous systems, a delay was present between the concentration in the blood and that measured by the interstitial fluid (ISF) of the subcutaneous sensor. There remains much to be done, however, as a truly long-term sensor must balance biofouling, accuracy, invasiveness, and reproducibility. Any subcutaneous sensor must be used with an algorithm to attempt to model the blood-glucose value from interstitial measurements, in order to achieve accurate insulin dosing.

Modern, implantable glucose sensors have tremendous potential, but technological limitations remain. The biocompatibility of biomaterials used for sensor implantation is one of the major barriers to long-term function. Implantable glucose sensor materials used to date are known to induce inflammation and lead to tissue capsule formation and sensor failure.[90] While implantable glucose sensors have great potential, existing sensors require regular replacement, with subcutaneous sensors lasting up to 7 days, though fouling of the sensor can reduce this to 2-4 days and some manufacturers recommend replacement after 3 days.[69, 94] Intravenous implantable needle-type sensors perform worse, lasting a maximum of 48 hours. Furthermore, implantable sensors must be repeatedly calibrated (as often as every 6 hours), undermining improved patient compliance.[95] The foreign body response to these implantable sensors results in changes in glucose diffusion to the sensor, leading to inaccurate readings.[90] Implantable sensors provide blood glucose readings, but to date have not been co-fabricated with a delivery system (other than via wiring to an externally controllable insulin pump).

In addition, a significant sub-population of diabetics are unable to get stable, durable glucose sensing from existing devices.[96] Current-generation sensors are also limited in their ability to recover from device failure. Most devices rely on single-sensor architectures and thus are more prone to catastrophic failure. This means that, should the single-sensor fail, there is no back-up sensor available to restore functionality to the device. Nor is there an array of sensors present to offer any sort of average readings. Reliance on a single-sensor system may prove ineffective for the realization of true long-term sensing. Redundancy of this sort is not inherent in current systems, an issue that requires attention for future-generation technologies.

Some novel attempts at solving the problem of biofouling for electrochemical sensors have taken a microneedle-based approach.[97-101] Microneedles have been developed for efficient transcutaneous delivery of a variety of drugs and therapeutics, including insulin (using programmable insulin pumps and a microneedle-based catheter, of sorts). These are metallic and polymeric delivery devices are non-invasive and painless to the patient, which can improve compliance amongst diabetics. These sensors are relatively non-invasive, and thus user-replaced sensor devices might be much easier to handle and much less painful for the patient.

Microneedles exist in a variety of architectures, depending on their purpose.[102] The diameter ranges from 50 to 150 μm at the base, with a length from microns to hundreds of microns, generally long enough to achieve transdermal penetration. To deliver therapeutics, solid needles are often coated with biodegradable polymers containing various therapeutics to release drug over time or are themselves biodegradable / erodable. Electrochemical sensors have been developed using hollow microneedles that pierce into the subcutaneous space and, through capillary action, wick interstitial fluid away towards an electrochemical sensor.[103-107] Narayan *et al*. have used carbon-fiber based sensors inserted into the cavity of hollow microneedles to accomplish direct-sensing of analytes such as peroxide and ascorbic acid, though they did not explore glucose sensing with this architecture.[103] Liepmann *et al*. have used the wicking process to draw interstitial fluid through the hollow

needle towards a flat sensor located on the other side of the silicon-based microneedle array. The system employs a dialysis membrane at the base of the microneedle. Dialysis fluid is flowed along a channel at the anterior of the array, which carries the analyte fluid through a diffusion barrier and on to the three-electrode GOx-based sensing component.[104]

Wang *et al*. take this one step further and have assembled an actual RF device for signaling the readings taken by such arrays.[106] These flat sensors are based on the same technology as glucose monitoring test strips and other implantable sensors, though they are treated for longer-term stability using many of the aforementioned coatings.

Microneedles, as a platform, offer some exciting possibilities for the future of CGMS. The advantages of such a non-invasive sensor, however, open up the possibility for enhancing patient comfort, affording continuous monitoring, and allowing easily-replaceable platforms for both short and long-term sensing needs.

4.1.3. Optical Detection of Glucose—Efforts towards non-invasive or minimally invasive glucose monitoring have focused on many optical sensors. Indeed, the first work on glucose monitoring was performed optically.[11] The changeover to the aforementioned electrochemical systems came after 1987, when Higgins et al. introduced a pen-sized monitor.[11] Optical methods were based on the GOx reaction, as well, but resulted in a change in the absorption characteristics of a dye immobilized on the test strip.[107] Since then, advances in the field have exploited fluorescence, infrared (IR), and Raman spectroscopy towards in vivo glucose sensing. These approaches have the potential to be coupled to an explicit closed-loop delivery system. This section highlights the most recent developments in the materials used for optical sensors.

Fluorescence-based Glucose Sensors: Fluorescence sensors represent the most pervasive type of optical sensor studied for glucose detection. Most are based on intensity variance caused by a change in glucose concentration. Significant work on boronic acid based small-molecule fluorescent probes for the detection of a variety of saccharides, including glucose, was recently reviewed by Wolfbeis and Mader.[80] Polymeric systems must combine affinity and permeability for glucose, a fluorescent moiety, and the ability to function in biological systems. Singaram et al. have used the quenching of an anionic dye with a viologen imbedded within a polymeric matrix.[108] The hydrogel scaffold was composed of poly(2-hydroxyethylmethacrylate) (pHEMA), which is a non-toxic system that is also not easily degraded (Figure 3(a)). When glucose binds to the PBA moiety, the viologen-dye complex dissociates and fluorescence recovery occurred. Up to 20% greater intensity was observed and the range of measurable glucose concentrations was from 2.5-20 mM (45-360 mg/dL). Though the system is still slightly more selective for fructose (2-fold), the relative concentration of glucose to fructose in the blood compensates for this difference. The same group also accomplished in vivo testing of an optimized version of the sensor. The preferred quencher:dye ratio was found to be 10:1 as this provided linear signals when subjected to biologically relevant glucose levels. The hydrogel-sensor mixture was adhered (with a common veterinary soft tissue adhesive) to a fiber tip and yielded continuous glucose readings for ten months.[109] An alternative approach was taken by Tao et al., who used polymers bearing PBA units and pyrene or naphthalene pendants. Glucose binding with the PBA units causes the polymer to rearrange in such a way as to align the pendant aromatic units. This overlap is detectable under UV irradiation.[110]

Fluorescence Resonance Energy Transfer (FRET) is also used for optical glucose detection. Klimant and Zenkl used fluorescent acrylamide nanoparticles bearing PBA units for this purpose.[81] A donor and acceptor were incorporated into the polymer of N,N'-methylenebis(acrylamide), 3-acrylamidophenylboronic acid, and N-isopropylacrylamide in

varying ratios. The hydrogel of this polymer swelled in response to glucose, which caused a change in the distance between donor and acceptor dyes. The fluorescence difference was correlated to glucose concentration. Willner et al. functionalized CdSe-ZnS quantum dots (QDs) in order to analyze the competitive binding of dopamine and saccharides, such as glucose.[111] Similarly, FRET efficiency was modulated in response to glucose concentration such that glucose binding disrupts the conjugation between QD donor and dye acceptor moieties.

Among early work done on IR-based glucose sensors was the analysis of EDTA blood by attenuated total reflectance infrared spectroscopy (ATR-IR) from Heise et al.[112] However, most work related to the infrared region is not carried out with FTIR or ATR-IR but rather fluorescence emission in the IR or near-IR (NIR) region. For example, Pitner et al. used excimers from thiol-reactive squarine molecules that emit above 650 nm and detected glucose via enhanced fluorescence intensity.[113] The NIR is a highly desirable region for sensing, primarily because there is little to no biological interference for signals beyond ~650 nm, making detection peaks in this area less noisy and more amenable to accurate and specific readings. Secondly, visible fluorescence often requires high-energy excitations, which may cause long term damage to tissues or sensing materials.

Much focus on NIR-fluorescent glucose sensors lies in single walled carbon nanotubes (SWNTs) because they do not photobleach, like many other dyes, and would thus form longer-lasting and more accurate sensor components. Strano et al. developed a system in which nanotubes were excited at 785 nm and fluoresced anywhere from 950 nm to 1300 nm, depending on the structure, orientation and modification of the nanotube. Potassium ferricyanide [$K_3Fe(CN)_6$] reversibly adsorbs to the surface of SWNTs and quenches or shifts fluorescence. Using nanotubes suspended in GOx solutions, they showed that relative fluorescence intensity increases upon sequential addition of glucose.[114] They also explored nanotube aggregation for glucose sensing, wherein phenoxy-derivatized dextran, which is a polysaccharide that acted as an analogue of glucose, was attached to the SWNTs. Using concavalin A (ConA), aggregation was found to occur and photoluminescence decreased. ConA has specific binding sites for sugars at physiological pH, which was used to induce aggregation of the dextran-coated SWNTs. Upon addition of controlled amounts of glucose (3-11 mM or 54-198 mg/dL), the aggregates dissociated because of competitive binding of glucose with ConA (Figure 3(b)).[115] Another novel system employed DNA-wrapped SWNTs for glucose detection. Karachevtsev et al. immobilized GOx on nanotubes and demonstrated that it retained its structure and function. Potassium ferricyanide was added to quench fluorescence (emission at 1146 nm), which was restored upon sequential addition of glucose.[116]

Raman Spectroscopy-based Glucose Sensors: Raman spectroscopy, more specifically surface enhanced Raman scattering (SERS), has been explored for its potential utility in glucose monitoring. Van Duyne et al. developed silver film nanosphere (AgFON) substrates which were coated with (1-mercaptoundeca-11-yl)tri(ethylene glycol) (EG3).[117] These EG3 layers were developed as mimics for the aqueous humor, the aqueous substance filling the space between the lens and the cornea, and formed 2 nm bilayers on the noble metal surfaces. EG3 partitions showed affinity for glucose and resisted fouling by protein or enzyme adsorption. The AgFON-EG3 was placed in physiological pH saline which contained 0-25 mM (0-450 mg/dL) glucose. SERS spectra were obtained for AgFON-EG3 alone, with albumin, and with each concentration of glucose. Subtracted spectra were compared to that of crystalline glucose and were found to match. The signal was also accurate according to the Clark Error Grid, a standard diagram metric for the accuracy of glucose sensors.[108] Van Duyne et al. have also created the first in vivo studies utilizing SERS.[118] They placed a similar AgFON-EG3 membrane subcutaneously in a rat and

measured the glucose concentration of the ISF via a specially designed viewing window. Barman et al. have sought to solve the issue of delayed glucose readings in the ISF as compared to blood. Using Raman spectroscopy, they have performed modeling and experimental measurements to calibrate and quantify this lag with a dynamic concentration correction (DCC) as an effort to improve the efficacy of implanted optical continuous glucose monitoring systems.[119] The advances made in Raman spectroscopic detection of glucose-in addition to other methods of glucose detection-will have important consequences for the development of a synthetic pancreas when combined with the controlled release of insulin from polymers.

4.2. Glucose-responsive Polymers for Regulated Insulin Delivery

4.2.1. Controlled release of insulin from polymers—Several reviews have been written describing the controlled release of insulin and other molecules in detail.[120-127] Early work on the controlled release of insulin from polymer matrices showed up in the literature in the early 1980s.[128, 129] This work employed the use of insulin, typically stored as particles in the size range of 10s to 100s of microns, trapped in ethylene-vinyl acetate (EVAc) copolymer pellets (on the millimeter or centimeter scale – see Figure 4(a)). The continuous release of insulin from these pellets showed the induction of normoglycemia in diabetic rats and the release kinetics were controlled by changing the solubility of the insulin, the loading of insulin within the matrix, and the porosity of the matrix. Although these devices worked well at inducing normoglycemia, it was recognized early that the continuous release of insulin was only a first step: “One could speculate that, with further development, these insulin + polymer implants might be used clinically in conjunction with dietary control or as an insulin depot linked to a regulatory feedback control with a glucose sensor.”[129] Later attempts at continuous insulin release used poly(lactide-co-glycolide) (PLGA)-poly(ethylene glycol) (PEG)-PLGA polymers. The release rate of insulin was controlled by varying the molecular weight of PEG and the ratio of DL-lactide to glycolide.[130] Additionally, chitosan microcapsules have been used as controlled release systems for insulin.[131] Chitosan was chosen because of its biocompatibility, biodegradability, and bioadhesive properties. Chitosan is not the only polysaccharide that has been chosen for the constant release of insulin, however. Starch, sephadex, sodium hyaluronate, ethyl(hydroxyethyl)cellulose, chitosan/calcium alginate mixtures, and hyaluronic acid have been used to encapsulate insulin.[132, 133]

An early attempt (1987) at actively controlling the release of insulin in response to external stimuli, such as the concentration of glucose (sometimes called a “smart” material) came by embedding magnets in the implanted EVAc pellets and exposing the rats to oscillating magnetic fields. Passive, continuous release of insulin resulted in a decrease in the blood glucose levels of the rats, in addition to a further reduction of nearly 30% by exposing the rats to an oscillating magnetic field.[6] This has more recently (2006) been extended to magnetite nanocrystals and insulin co-encapsulated into PLGA microparticles,[134] and charge-coupled insulin loaded PLGA microparticles and micromagnets (2008).[135] A significant difference between the 1987 and the later studies is that the polymer was surgically implanted in the earlier study; in the later study the polymer microparticle were orally administered. However, these approaches suffer from the fact that the subject must be in a magnetic field to control insulin release.

Additional smart materials that have proven to be useful in the actively controlled release of insulin are materials that are sensitive to pH and temperature.[136-138] In one study, the insulin loaded EVAc matrices were loaded with Sepharose beads that had GOx immobilized on their surface. When glucose in solution entered the polymer matrix, gluconic acid was produced, causing a drop in the local pH. This fall in pH caused the solubility of insulin to

increase, thereby increasing the amount of excreted insulin from the matrix.[139] In a separate study around the same time, it was found that the simultaneous release of somatostatin with insulin extended glycemic control in diabetic rats, as opposed to the release of insulin alone (Figure 4(b)).[140] Other techniques used to control glucose levels include low-frequency ultrasound to transdermally deliver insulin, referred to as low frequency sonophoresis (LFS). This approach is similar to injecting insulin, but without the pain and possibility of infection associated with injections.[141] Along those lines, it was shown that LFS can be used to extract ISF, which can be used to monitor blood glucose levels.[22] Later work showed that acoustic cavitation, defined as the acoustically induced activity of gas filled cavities, including nucleation, oscillation, and collapse, is the key mechanism of skin permeabilization during LFS.[142] Similar to transdermal delivery, it was discovered that insulin could be delivered through inhalation by designing large (>5 μm) and porous (<0.4 g/cm^3) particles. This pulmonary drug delivery offers many of the same advantages as transdermal insulin delivery.[143-145] Pulmonary delivery was also demonstrated with a nebulation of solid insulin proteins suspended in ethanol.[146] Finally, in the last decade or two, science and engineering has observed a revolution at the micron- and nano-scale. Drug delivery, in this case insulin, has been strongly influenced by these revolutions, and the reader is encouraged to examine these articles.[147-149]

In addition to delivering insulin from an implantable device, the stability of stored insulin is a major concern in designing a long lasting polymeric insulin depot. It was first recognized as early as 1944 that insulin can aggregate and form fibrils.[150, 151] These fibrils and other types of aggregates are now recognized to have different biological activities, and hence cause problems with insulin loaded implants, than the monomeric insulin form.[152] Langer and coworkers began examining this problem in 1991, attempting to define a molecular mechanism and corresponding kinetic scheme for insulin aggregation upon agitation in the presence of hydrophobic surfaces (Figure 5).[153] This study demonstrated that monomeric insulin stability was enhanced at higher insulin concentration, and attempted to use the proposed kinetic scheme to explain this effect, which was that agitation was necessary to induce aggregation. This work was extended to the air-water interface and the hydrophilic glass-water interface,[154] recognizing that differences exist in the structure of adsorbed biological molecules at hydrophilic and hydrophobic interfaces.[155] The study showed that the addition of nonionic detergents known as excipients strongly reduced the amount of insulin aggregation. In 1993, Langer and coworkers demonstrated the controlled release of recombinant bovine somatotropin and zinc insulin from hydrophobic poly[1,3-bis(*p*-carboxyhydroxy)hexane anhydride] with sucrose as an excipient.[156] The released proteins appeared to maintain their integrity over a 3 week release period. The important conclusion of this study was two-fold: (i) insulin stability could be increased by the use of a hydrophobic degradable polymer, namely a polyanhydride, and (ii) excipients could be incorporated into this polymer, leading to increased stability of the deliverable protein. These results were further explored by examining the deterioration of lyophilized insulin in the presence and absence of water.[157] Future work will in this field should focus on the development of novel excipients,[158] design of new supramolecular insulin analogs,[159] and examining different insulin crystal isomorphs (similar to the methodology presented in reference[160]) or naturally occurring supramolecular assemblies.[99]

4.2.2. Responsive hydrogels for controlled insulin release—In addition to erodible polymers, polymeric gels loaded with therapeutics such as insulin that respond to changes in concentration of glucose have emerged as a promising material for treatment of diabetes. These polymeric materials are sometimes referred to as “smart” or “intelligent” materials. Due to the large amount of work in this area, we will focus on a few examples.

Smart materials based on the sensing of glucose using PBA, have been investigated, as well as their potential to transduce sensing into a release of insulin. When in water, PBA (and its derivitatives) exist in an equilibrium between uncharged and negatively charged forms. Glucose can bind to the anionic PBA. (Figure 6(a)) When PBA is made into a gel, this binding of glucose can give rise to a reversible volume change of the gel—a so called "volume phase transition"—thought to be the result of a change in the counterions' osmotic pressure in the gel. This volume change could allow for the release of insulin from the gel. In 1991, PBA was first used in a copolymer (with poly (N-vinyl-2-pyrrolidone) (PNVP, PVP, or NVP)) by Kitano et al. to sense glucose.[161] This polymer, poly(NVP-co-PBA) was shown to bind to both PVA and glucose by monitoring the change in viscosity of the copolymer as PVA or glucose was added.[161] This work was extended the following year by changing the polymer molecular weight and concentration which allowed for changes in the polymer viscosity.[161] In this work, it was suggested that a responsive system could be created that utilized competitive binding between PVA and glucose. One difficulty with this system, however, is that the PBA-polymer complex can be formed only under basic conditions, which limited practical use.[161] The first attempt to use this system under physiological conditions (here pH 7.4 and room temperature) was performed by adding amino groups to a PBA based copolymer.[162] This polymer, called BAP, (poly(acrylamidophenylboronic acid-co-N,N-dimethylaminopropylacrylamide-co-acrylamide-co-N,N'-methylenebis(acrylamide)) contained amino groups that would coordinate to boron atoms, allowing for the formation of a complex with glucose under physiological conditions.

In another study, gel beads containing PBA were synthesized, and the association constants between PBA groups and several hydroxyl-containing molecules were found (at pH 8.5). The affinity series went alkyldiols < glucose < open-chain saccharide isomers.[163] This study also presented a new idea: a gluconic acid modified insulin (G-Ins) could be synthesized and bound to PBA. This G-Ins would come off the hydrogel beads in response to competitive binding with free glucose in solution, thus demonstrating the possibility of a glucose responsive insulin release gel.[163] This work was extended to amine containing PBA gels that could respond to glucose challenges at pH 7.4 for over 120 hours.[164] Around the same time, the synthesis and characterization of a another copolymer, DB-15, which is N-N dimethylacyrlamide containing 15 mol % 3-(acrylamido)phenylboronic acid (APBA) was reported.[165] This polymer showed a shift in its lower critical solution temperature (LCST) in response to the glucose concentration.[165] This work was later extended to copolymers of poly(*N*-isopropylacrylamide) (PNIPAAm), PBA, and N—(3-dimethylaminopropyl)acrylamide (DMAPAA), which showed large shifts in the LCST in response to glucose concentration changes at pH 7.4.[166]

Furthermore, the feasibility of a glucose sensing system based on a change in the fluorescence during competitive binding between a fluorescent diol compound—6,7-Dihydroxy-4-methyl-coumarin—and glucose toward a PBA compound was demonstrated.[167] This was an important step towards making an all synthetic opto-sensing system for glucose.[167] Similar work allowed for an electrochemical measurement of glucose by synthesizing a PBA copolymer-PVA complex that changed its swelling degree in response to glucose concentrations specifically.[168] The PBA copolymer-PVA complex was coated on a platinum electrode. Glucose addition to the solution that was in contact with the electrode caused a swelling of the PBA copolymer-PVA complex, allowing for increased diffusion of ion species and hence a measurable current.[168] The kinetics of complexation of these PBA copolymer-PVA complexes were examined in a subsequent publication.[169]

In 1998, Kataoka et al. reported the development of a gel made from PNIPAAm containing some 3-acrylamidophenylboronic acid (AAPBA) that could swell in response to changing

glucose concentrations. The binding of glucose to the AAPBA moiety causes the gel to become “more hydrophilic, and with a concomitant increase in ion osmotic pressure due to the counter-ions the phase-transition temperature of the gel shifts to higher value in the presence of glucose compared to the condition without glucose.”[170] This led to a swelling of the gel. The authors then demonstrated that if the polymeric gel is loaded with insulin prior to glucose exposure, the gel can act as a depot, releasing more insulin into the surrounding solution when glucose is present than when glucose is absent. In addition to demonstrating how to transduce a chemical signal, namely glucose concentration, to the release of insulin, this system is completely synthetic. Biological systems may suffer from issues such as stability, toxicity, and immunogenicity to a greater degree than some synthetic systems. One disadvantage of this work was that it was performed at pH 9.0. Additional review of this work can be found in a number of publications.[171-173]

Since this early work, there has been subsequent work to achieve simultaneous sensing of glucose and controlled release of insulin under physiological conditions. In one study, copolymers of 4-(1,6-dioxo-2,5-diaza-7-oxamyl)phenylboronic acid (DDOPBA) and PNIPMAAm were fabricated. Glucose- and pH- dependent changes in the critical solution behavior of these copolymers were investigated at varying temperatures, revealing definite glucose sensitivities near physiological conditions.[174] This work was extended to demonstrate glucose dependent volume changes at physiological conditions.[175] Additionally, the swelling kinetics of similar gels have been studied in detail.[176] More recently, a copolymer containing DDOPBA, PNIPMAAm, and CIPAAm was synthesized. The CIPAAm unit was added to control the hydrophilicity of the gel, without influencing the sharpness of the volume phase transition.[177]

More recently, Kataoka and coworkers have developed PBA copolymers containing N-isopropylacrylamide (NIPA-co-AAPBA) that serve as the basis for a colorimetric glucose assay.[177, 178] This was accomplished by the fabrication of a structurally colored—a color that arises from physical optics such as the Bragg diffraction of light—gel of NIPA-co-AAPBA. A periodically ordered interconnecting porous gel exhibiting the desired structural color was prepared by using a colloidal silica crystal as a template. These gels are responsive to a variety of environments and designed to be green in the presence of low concentrations of glucose (<7.8 mM), red in the presence of high concentrations of glucose (>11.0 mM), and yellow at intermediate concentrations.[178] This was later extended to glucose concentrations between 5 and 8 mM.[179]

In similar studies by the groups of Siegel and Ziaie,[171, 172, 180] the authors incorporated a hydrogel containing a PBA moiety, 3-methacrylamidophenylboronic acid, MPBA and acrylamide, AAm into a microfluidic glucose sensor and potential insulin delivery system. In their system, they observed glucose induced swelling at pH 7.4, but not to the degree that glucose induced swelling was observed at pH 10. The authors attribute the swelling behavior of this hydrogel in part to glucose induced cross-linking of the MPBA moieties, although the exact mechanism needs an improved theory to aid in a quantitative description of the phenomena. In conclusion the authors state, “further work on microfabricated glucose sensors and closed-loop insulin delivery systems will require a close interplay between polymer chemistry, an understanding of the physical chemistry of hydrogel swelling as a function of glucose concentration and pH, and improved micro-and nanofabrication techniques.”[173]

In addition to phenylboronic acid based systems, a combination of dextran and ConA can be used to sense glucose. In this scheme, ConA can bind to four dextran molecules (a molecule that can bind to many ConA molecules) at low glucose concentrations. However, glucose and dextran can both bind to ConA, and this competitive binding leads to ConA being bound

to four glucose molecules at high glucose concentrations. Hence, at low glucose concentrations, the solution contains highly crosslinked dextran and ConA and is highly viscous; at high glucose concentrations, the individual ConA molecules bind to four glucose molecules each and the solution has a low viscosity (Figure 6(b)).[181-183]

5. Biocompatibility of materials

Biocompatibility of the polymers used for artificial pancreases, synthetic pancreases, and sensors has been cited as a concern regarding the viability of the device.[32, 184] Imaging methodologies based on fluorescence and bioluminescence have recently been developed to assist in biocompatibility assessment.[39, 53, 183, 185, 186] The fluorescence based reflectance screening operates through the use of a probe molecule, ProSense-680. This molecule has fluorophores attached to it, which quench each other in the intact state. Upon cleavage by an enzyme, the fluorophores are separated and the molecules fluoresce. The enzyme responsible for cleaving ProSense-680 is cathepsin, which is a proteolytic enzyme released from neutrophils during phagocytosis.[35] The bioluminescence based assay uses luminol – a molecule that luminesces in response to reactive oxygen species – to probe for biocompatibility. Macrophages, neutrophils, and fibroblasts release reactive oxygen species in response to foreign bodies, as in the case of polymer implantation. Through bioluminescent screening, the foreign body response to implanted materials can be visualized in real time.[185] These modalities have enabled for drug screening[185] and screening polymeric libraries.[186] These technologies present a promising possibility for analyzing large libraries of biomaterials to determine which are biocompatible. Histology is still a necessity, as neither of these methodologies is capable of providing the depth of information that histopathology can. Imaging developments, such as the fluorescent and luminescent techniques outlined above, illustrate an open avenue of research to be explored, which is the development of new methods capable of assessing biocompatibility information in real time and in vivo.

6. Conclusions and Future Directions

Several challenges lie ahead in the development of the artificial pancreas. Inducing normoglycemia in a diabetic mouse through xenotransplantation of rat islets encapsulated in alginate has been demonstrated.[187, 188] There has been less success reported in higher order mammals, such as non-human primates, when they are treated for diabetes with encapsulated xenogenic islets.[188] Generating new materials that conform to the criteria outlined above – biocompatibility and ability to freely diffuse insulin, nutrients, and waste products – may further reduce biofouling complications that arise when transplanting encapsulated materials in primates.

One of the limits in translating islet encapsulated devices from the bench top to clinical trials rests in the reliability of mouse models. To date, it has been difficult to translate positive results from rodents to primates in this area. Additional work on modeling the fibrotic response to implanted devices in both rodents and primates will be necessary to allow preclinical development of these types of systems.

Electrochemical glucose detection has offered the most accurate and most rapid information regarding blood-glucose concentration. It is specific for glucose and highly sensitive. Electrochemical sensors could provide this level of detail continuously, if implanted in a living organism. The major disadvantage to using these sensors in vivo, however, is their tendency to succumb to variations in signal due to biofouling. Significant future efforts in this field will reside in the development of better and longer-lasting isolation methods (new polymeric coatings, for example) that protect the sensor from deleterious biological agents while still allowing diffusion of analyte and fast response speeds. Further, more accurate

algorithms, or the ability to continuously measure blood glucose must be developed for an optimal closed-loop insulin delivery mechanism to be realized.

Optical glucose sensing offers the distinct advantage of being able to detect glucose in a wholly non-invasive manner, which would lead to enhanced patient comfort and reduced chance of infection. These methods of analysis are becoming more accurate and specific for glucose, however they have not yet attained the levels of their electrochemical counterparts. Recognition of glucose irrespective of other similar sugars present will remain a challenge, as will signal enhancement and sensitivity. The complex nature of signal deconvolution must also be solved if a simple, user-friendly device is to be envisioned from these technologies.

The completely artificial pancreas holds a number of advantages over biologically based materials for diabetes treatment, namely the elimination of immunological responses arising from biological contaminants. However, several challenges remain in the implementation of a functioning artificial pancreas. One is the engineering of a device with a reasonable ratio of deliverable insulin to polymer scaffold. To make a device that will work for a period of months, a large amount of insulin must be stored in the device, or simple refilling must be possible. This will only be practical if the ratio of insulin to polymer is such that a large mass of polymer is not necessary for implantation. Additionally, improved biocompatibility may be necessary for these types of devices to function in vivo for a long period of time.

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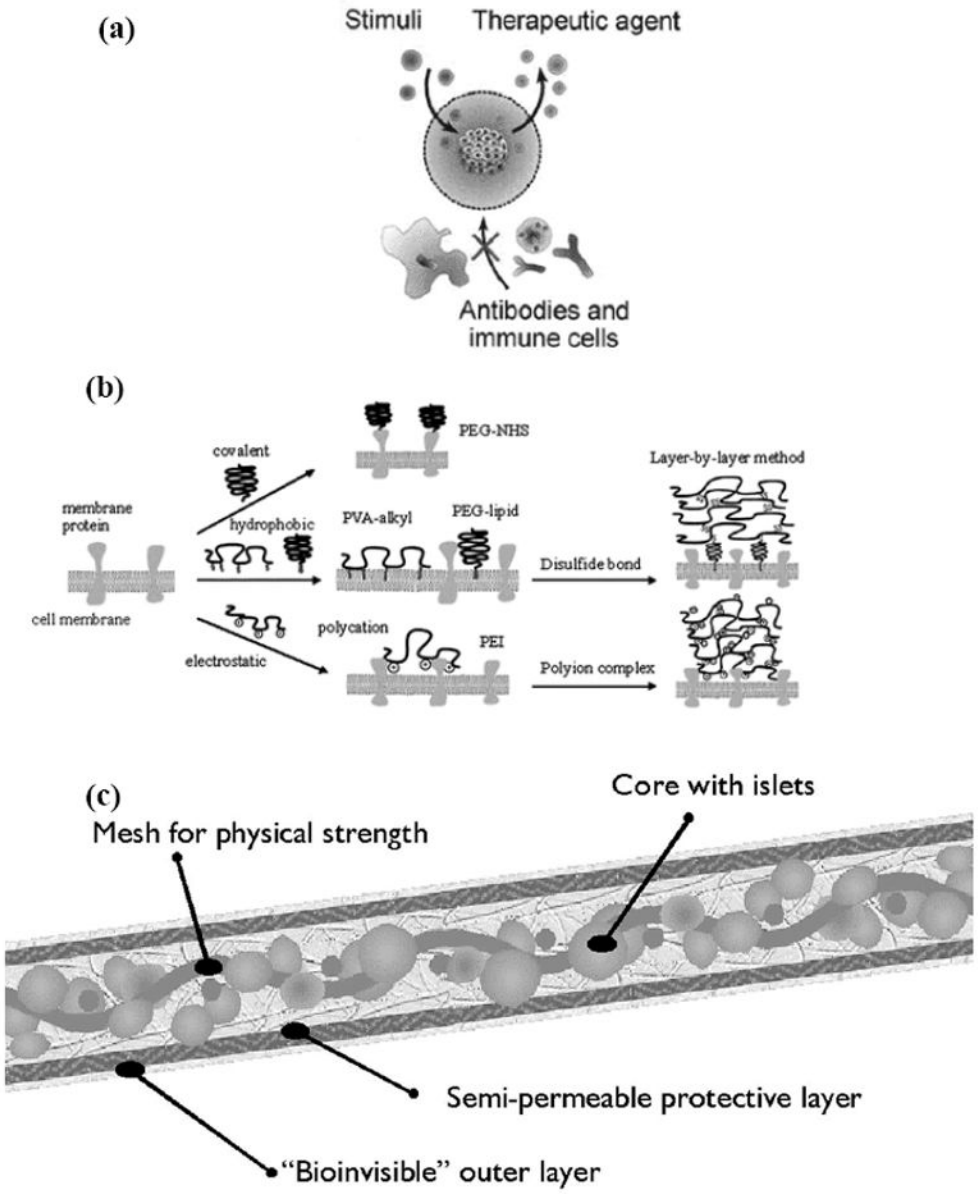

Figure 1.

(a) Microencapsulation of islets in which nutrients, oxygen and stimuli diffuse across the membrane, whereas antibodies and immune cells are excluded. (Reprinted by permission from Macmillan Publishers Ltd: Nature Medicine, copyright (2003)).[189] (b) Schematic illustration of surface modification of a cell with synthetic polymers by covalent bonding, hydrophobic interaction, electrostatic interaction, and the layer-by-layer method. (Reproduced by permission of The Royal Society of Chemistry from reference[56]). (c) Diagram of an Islet Sheet, in cross-section. Alginate, containing up to 40% (v/v) islet tissue, is sandwiched between acellular alginate layers and gelled. A polymer mesh can be included in the islet-containing core to provide physical strength. Currently the semipermeable and bioinvisible layers are one and the same. Typical Islet Sheets measure 4 cm × 8 cm × 250 μm. (Reproduced with permission from reference[63]).

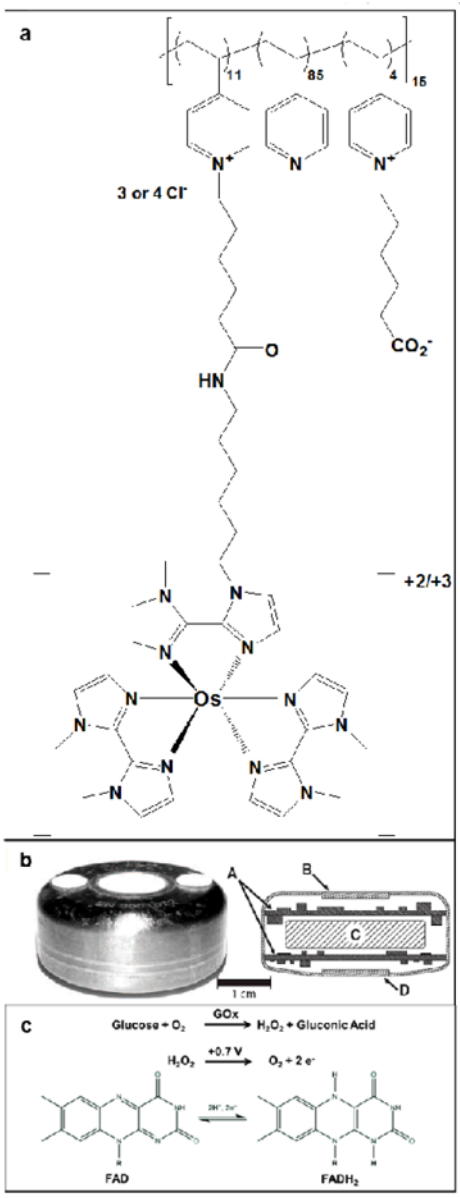

Figure 2.

(a) Chemical structure of an inorganic redox center pendant on an organic polymer backbone. This system is used as an electrochemical mediator for the sensing of glucose via GOx. The electron transfer accompanying the reduction/oxidation of the FAD cofactor can be monitored by an electrode using such mediators (redrawn from reference).[83] **(b)** Schematic diagram and image of an implantable subcutaneous continuous glucose monitoring system. A = electronic components; B = location of telemetry circuits; C = battery; D = sensor array. Reproduced from reference.[93] **(c)** Chemical structure of FAD and $FADH_2$ as well as the basic redox mechanism of GOx sensors.

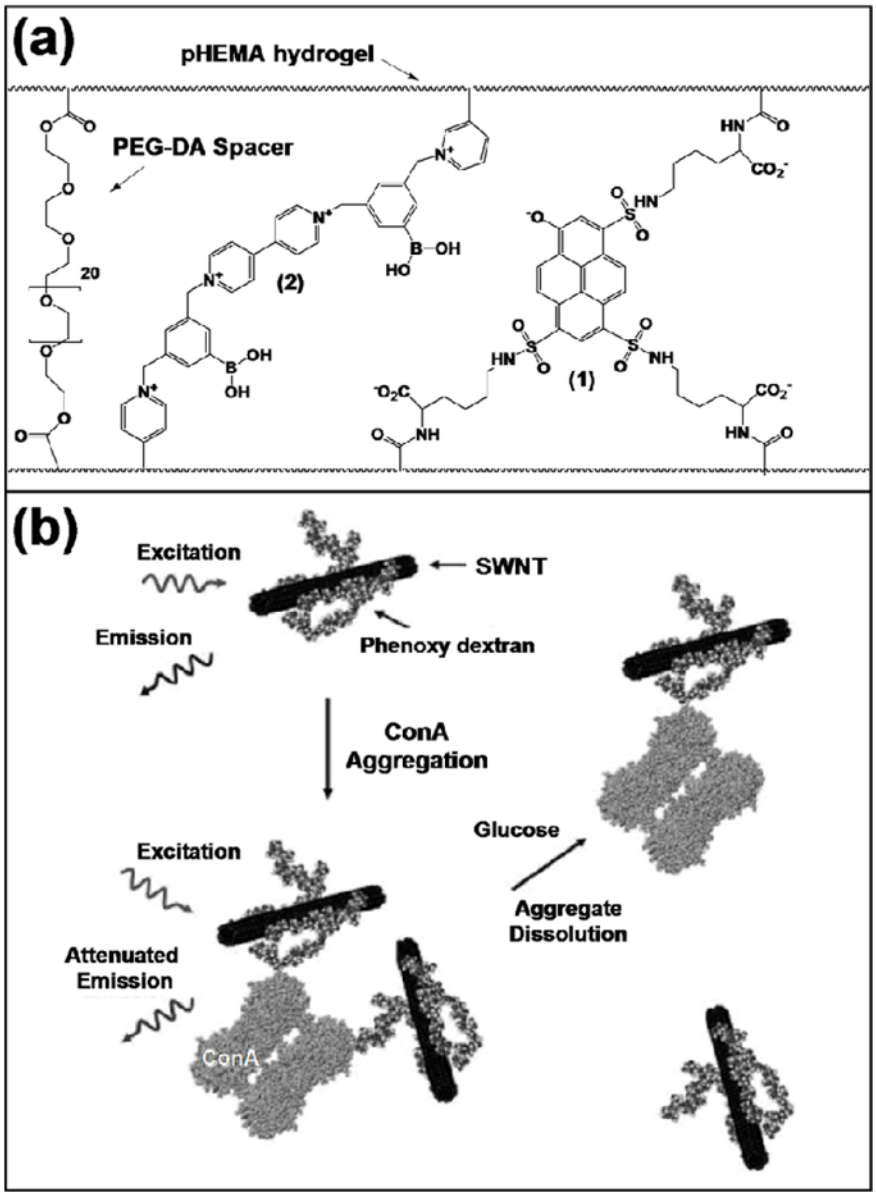

Figure 3.

(a) Chemical representation of a p(HEMA) hydrogel scaffold, with polyethylene glycol dimethacrylate (PEG-DA) spacer, containing a fluorescent anionic dye (**1**) quenched with a viologen (**2**) due to electrostatic interactions. The PBA moieties bind with glucose and cause a separation of the dye-viologen complex, resulting in enhanced fluorescence (Reproduced by permission of the Royal Society of Chemistry from reference[108]. (b) Schematic diagram of reversible aggregation in SWNTs based on a glucose binding event. Phenoxy dextran-modified SWNTs agglomerate in the presence of ConA, causing quenched NIR fluorescence. Glucose competitively binds with ConA, dissociating the aggregates, and increasing fluorescence intensity (Copyright Wiley-VCH Verlag GmbH & Co. KGaA; Reproduced with permission from reference[114].)

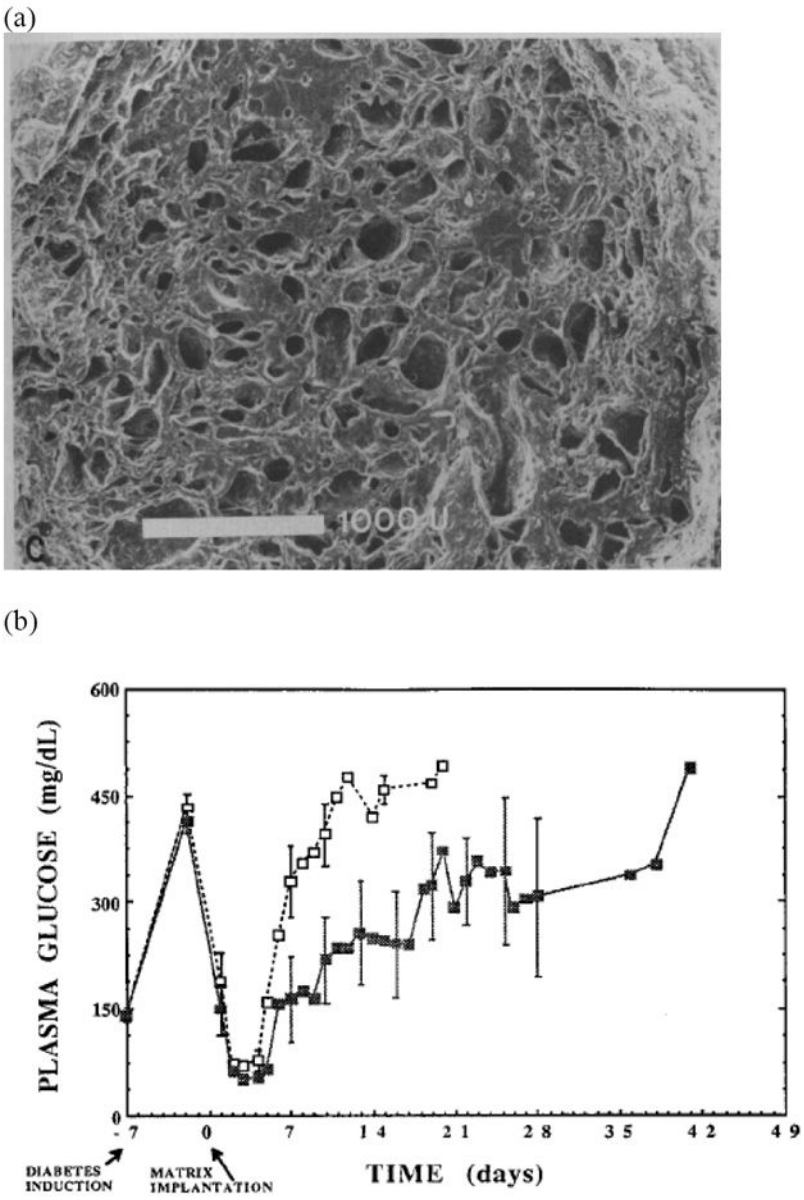

Figure 4.

(a) A SEM image of a section of insulin-containing EVAc matrix after the release of 98% of the enclosed insulin. The white scale bar refers to 1000 μm. (Reproduced from reference[128]. Copyright 2009 American Association – from Diabetes, Vol. 35, 1986; 684-691. Reprinted with permission from The American Diabetes Association). (b) Plasma glucose plotted against time for animals made diabetic and treated either with EVAc matrix release of insulin alone (□) or insulin with somatostatin (■). Plasma glucose rose threefold with streptozotocin induction of diabetes and fell with matrix implantation. The rats treated with insulin alone were hypoglycemic for the active portion of their therapy whereas the somatostatin group exhibited more physiologic glucose concentrations for much longer periods of time. (Copyright Wiley-VCH Verlag GmbH & Co. KGaA; reproduced with permission from reference[140]).

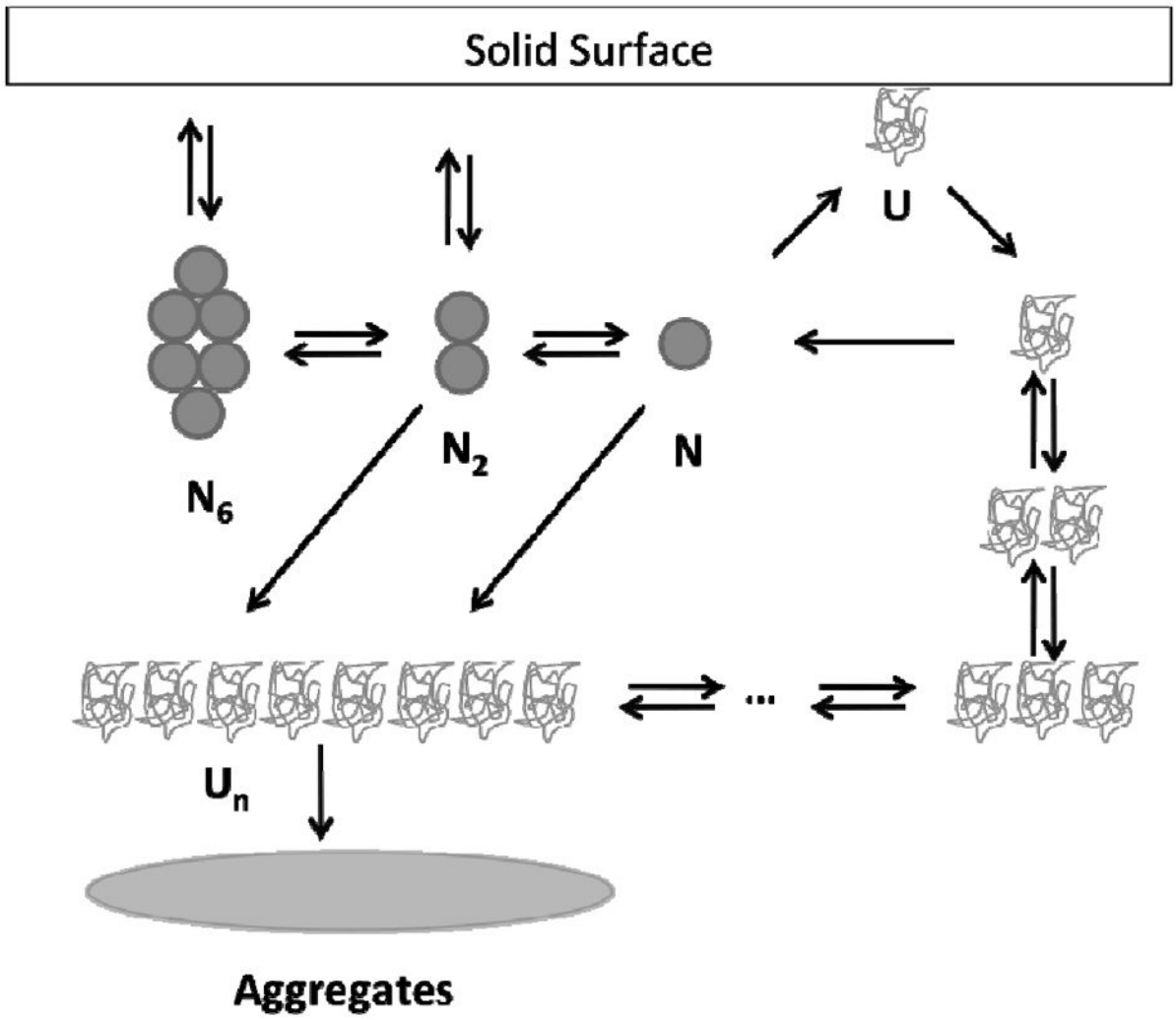

Figure 5.
Schematic representation of the proposed mechanism of insulin aggregation. Here, N stands for native insulin and U stands for unfolded insulin. (Copyright Wiley-VCH Verlag GmbH & Co. KGaA; reproduced with permission from reference[154]).

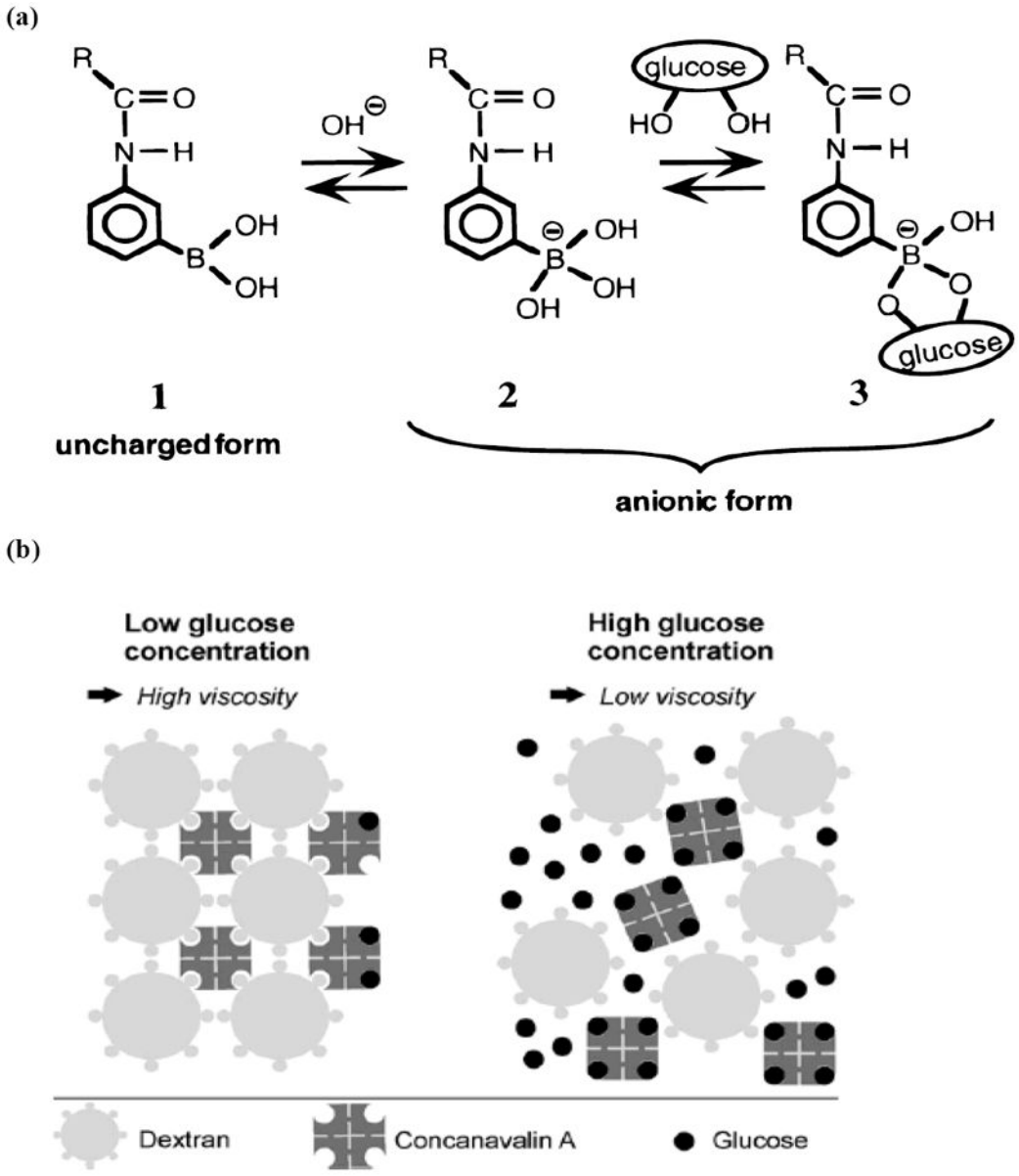

Figure 6.

(a) Equilibria of (alkylamido)phenyl boronic acid. The presence of glucose shifts the equilibrium to the right, causing the polymer to become more hydrophilic. This shift changes the swelling degree of the polymeric gel, causing entrapped insulin to be dissolved in solution. (From reference[170]. Reprinted with permission from the Journal of the American Chemical Society © 2000 American Chemical Society). (b) Principle of the competitive binding of glucose and dextran on ConA in the case of low and high glucose concentrations. (Reprinted from Colloids and Surfaces B – Biointerfaces, Vol. 76, R.X. Lin, J. Han, J. F. Zhang, J. Nie, "Glucose-responsive composite microparticles based on chitosan, concanavalin A, and dextran for insulin deliver," page 483-488, 2010, with permission from Elsevier. From reference[181]).

Table 1

Summary of several approaches to diabetes therapeutics, with advantages and disadvantages of each.

Approach	Description and Types	Advantages	Disadvantages
Open-Loop Insulin Therapy	Externally triggered insulin release (subcutaneous injection, insulin pumps, magnetic, ultrasound, iontophoresis)	User control; accuracy	Requires patient and/or physician programming and dosing calculations; invasive and often painful; user non-compliance
Islet Encapsulation	Hydrogels laden with living β-cells which sense and deliver insulin	Closed-loop; self-regulated control without external input	Long-term biocompatibility; scale up; islet positioning
Conformal Coatings and Planar Sheets	Thinner coatings on living β-cells which sense and deliver insulin	Enhanced strength, less of a barrier between encapsulator and cells; smaller injection volumes	More complex preparation; long-term biocompatibility
External Glucose Sensing	Finger-prick amperometric blood glucose test strips	Extremely accurate and reliable glucose readings; used to determine insulin dosing; affordability	Invasive, painful, requires repetition, lowered user compliance; sporadic monitoring
Internal Glucose Sensing	Wire or chip based amperometric sensors	Accurate glucose levels; continuous monitoring; potential to wire to insulin pumps towards closed-loop systems	Imperfect algorithms to predict insulin needs; long-term biocompatibility
Optical Glucose Sensing	Visible-light based measurements of glucose	Non-invasive	Sensitivity and accuracy; sporadic monitoring; synthetic difficulty
Polymer Delivery	Continuous, basal insulin release from polymer systems	Able to induce normoglycaemia	Not externally triggerable or glucose responsive
Smart Hydrogels	Reversible swelling triggered by glucose results in insulin release	Insulin release mimics that of β-cells; sensitivity and selectivity for glucose	Speed of response; long-term biocompatibility; drug dose
Smart Complexes	Reversible aggregation triggered by glucose causes insulin to diffuse	Faster response time as compared to hydrogels; sensitive; selective for glucose	Long-term biocompatibility; drug dose; toxicity