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## Recent Advances in Nanotechnology for Diabetes Treatment

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### Abstract

Nanotechnology in diabetes research has facilitated the development of novel glucose measurement and insulin delivery modalities which hold the potential to dramatically improve quality of life for diabetics. Recent progress in the field of diabetes research at its interface with nanotechnology is our focus. In particular, we examine glucose sensors with nanoscale components including metal nanoparticles and carbon nanostructures. The addition of nanoscale components commonly increases glucose sensor sensitivity, temporal response, and can lead to sensors which facilitate continuous *in vivo* glucose monitoring. Additionally, we survey nanoscale approaches to “closed-loop” insulin delivery strategies which automatically release insulin in response to fluctuating blood glucose levels. “Closing the loop” between blood glucose level (BGL) measurements and insulin administration by removing the requirement of patient action holds the potential to dramatically improve the health and quality of life of diabetics. Advantages and limitations of current strategies, as well as future opportunities and challenges are also discussed.

### Keywords

Nanotechnology; Glucose Sensors; Insulin Delivery; Nanomedicine; Diabetes; Nanomaterials

### Introduction

Diabetes is a metabolic disease characterized by chronically elevated blood glucose levels (BGLs) and an inability to maintain BGL homeostasis<sup>1, 2</sup>. Individuals with type 1 diabetes

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cannot produce insulin as a result of autoimmune destruction of the insulin producing cells within the pancreas, known as beta cells<sup>3</sup>. Type 2 diabetes is characterized by insulin resistance, or a deficiency in cellular response to insulin in the bloodstream<sup>2</sup>. In both cases, the loss of homeostasis-regulation mechanisms can lead to chronically high and low blood glucose levels known as hyperglycaemia or hypoglycemia<sup>3</sup>. Chronic hyperglycaemia can lead to a variety of symptoms including cardiovascular and neurological complications<sup>4</sup>, while hypoglycaemia can lead to lack of energy, unconsciousness, and death<sup>5</sup>. Diabetes has grown to become one of largest public health challenges globally, affecting 25.8 million in the United States and 382 million worldwide; and this number is expected to grow to 592 million by 2035<sup>5, 6</sup>. Furthermore, diabetes is expected to become the seventh largest cause of death worldwide by 2030<sup>4</sup>. The current standard of care for type 1 and advanced type 2 diabetics involves daily subcutaneous insulin injections, and frequent finger pricks to draw blood for the measurement of BGLs<sup>5</sup>. Daily insulin injections are painful and lead to patient noncompliance, and can lead to dangerous insulin overdoses<sup>5</sup>. Additionally, periodic measurement of blood glucose may not detect large fluctuations in BGLs which occur between points of measurement. Therefore, systems which improve blood glucose monitoring, or “close the loop” between glucose measurement and insulin delivery, are highly desirable.

The application of nanotechnology to medicine holds many possible advantages, such as access to small and clinically relevant areas of cells and analysis of small volumes of analytes. Additionally, the emergence of quantum effects leads to interesting and useful physical properties; for example, nanoscale carbon is stronger than steel, highly malleable, fluorescent, and exhibits excellent electrical conductivity<sup>7</sup>. Figure 1 displays typical research themes using nanotechnology for diabetes treatment. For example, the improved glucose sensor technology has an immediate and significant impact on the health of diabetics, as improved sensing will lead to more accurate insulin dosing and diabetes management. Indeed, advances in nanomedicine have already facilitated novel sensors which are capable of more frequent and convenient blood glucose measurements<sup>6, 8–12</sup>. Nanomedicine has also enabled more robust insulin delivery systems that can detect fluctuations in BGLs and automatically modulate the rate of insulin release to maintain normoglycemia<sup>6, 13</sup>. Such systems represent a tremendous advancement over contemporary standards of care. The clinical application of these technologies will allow diabetics to manage their disease more effectively and improve their health and quality of life.

## Nanotechnology Enabled Glucose Sensing

Accurate and frequent glucose measurements are the basis of contemporary diabetes management. However, it is commonly acknowledged that contemporary clinical glucose measurement systems are a nuisance to the patient as a result of frequent and painful needle sticks, and the current standard of intermittent testing can miss dangerous fluctuations in blood glucose concentration<sup>7, 14</sup>. Therefore, one of the most significant challenges in diabetes research is the development of glucose sensors which achieve accurate glucose measurements painlessly and frequently, with the goal of continuous glucose measurement. A wide variety of glucose sensing modalities have been reported in the last two decades, and commonly employ Concanavalin A (Con A), phenylboronic acid (PBA), or most commonly,

Glucose Oxidase (GOx) as a sensor for detecting glucose in solution. Common interfering species in blood and other bodily fluids include Uric and Acetic acids, as well as other carbohydrates such as Fructose, Lactose, and Sucrose<sup>6, 11, 15</sup>. Specificity for measuring only Glucose in solution requires the use of glucose specific enzymes, or the use of chemical or physical barriers with other detection modalities<sup>16</sup>.

Broadly speaking, glucose measurement systems can be classified into two categories: electrical and optical. A common mechanism of glucose detection involves using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) or a similar reduced species as a chemical intermediary which drives the reduction of another species. This in turn generates a measurable signal such as an increase or shift in fluorescence<sup>6, 8, 11</sup> or a change in current through an electrode of an amperometric sensor<sup>17–19</sup>. Advantages of hydrogen peroxide based detection schemes include relatively straightforward sensor operation and characterization via amperometric techniques, but disadvantages include the degenerative effects of hydrogen peroxide on the sensor, the relatively high electrical potential required to catalyse hydrogen peroxide, and the possibility of sensor interference<sup>20–22</sup>. As a result of the importance of quantifying the concentration of hydrogen peroxide in solution to other fields, many electrical-based H<sub>2</sub>O<sub>2</sub> sensors have been developed or adapted for glucose measurement. A summary of nanotechnology enabled glucose sensing technologies is included in Table 1.

### Electrochemical Glucose Measurement

The excellent conductivity and catalytic ability of nanoscale carbon structures has led to their use in a variety of glucose sensing modalities<sup>23–29</sup>. Additionally, electrically coupling glucose oxidase to nanoscale carbon structures modulates the electrical resistance of the structures<sup>24</sup>. The first to publish research on using a single carbon nanotube as a biosensor was Besteman, et al., who immobilized GOx on a semiconductor carbon nanotube *via* 1-pyrenebutanoic acid succinimidyl ester, and found that the conductivity of the nanotube was correlated to the activity of the immobilized GOx's response to the addition of glucose<sup>24</sup>. The addition of 0.1 mM glucose solution resulted in an approximate 10% increase in conductivity of the GOx-nanotube complex<sup>24</sup>. The system also exhibited a strong sensitivity to the pH of the surrounding solution<sup>24</sup>.

In most electrochemical methods, glucose concentration measurements are achieved by measuring an enzyme's activity either directly by coupling it to an electrode or indirectly by measuring changes in concentration of the reactants or products. An advantage of coupling this technique with nanoscale components is that nanostructures such as carbon nanotubes<sup>24, 26, 27, 29</sup> or gold nanoparticles<sup>17, 18</sup> are excellent catalysts for reducing H<sub>2</sub>O<sub>2</sub>. Furthermore, the high surface area to volume ratio of nanostructures increases the reaction kinetics. Lin and co-workers applied the technique of hydrogen peroxide reduction to electrochemically detect glucose<sup>25</sup>. GOx was directly immobilized on carbon nanotubes by forming amide linkages between GOx's amine residues and carboxylic acid groups on the carbon nanotubes *via* 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysulfosuccinimide<sup>25</sup>. At low operating voltages (–0.2 V), the H<sub>2</sub>O<sub>2</sub> generated by GOx action could be efficiently catalysed by the nanotubes, resulting in current flow proportional to glucose concentration. A linear response from 0–30 mM was found, and a small limit of detection of

0.08 mM was reported. As opposed to large glucose sensing electrodes, the use of an array of many nanoscale electrodes increases the signal-to-noise ratio and the sensitivity of the sensor by increasing its functional surface area. Additionally, the use of small negative potentials can reduce the risk of interference from unwanted redox reactions which occur at higher potentials. It has also been demonstrated that combinations of nanoparticles work together synergistically to realize more efficient hydrogen peroxide catalyzation than can be realized with any single component. Shan et al. found that the combination of graphene, gold nanoparticles (AuNPs), and GOx within a nanocomposite material can cause an increase in the electrocatalyzation current of hydrogen peroxide and oxygen as a result of synergistic interactions between graphene and AuNPs<sup>17</sup>. They developed a glucose sensor based on this technology which could operate at low potential (−0.2 V) and was capable of accurately measuring human blood glucose concentrations from 2.5–7.5 mM<sup>17</sup>.

Yang and co-workers (Figure 2) compared similar micro- and nano-scale GOx electrodes, and found superior performance from the nanoscale sensors<sup>30</sup>. The micro scale electrode was based on a bulk conducting polymer film with entrapped GOx, and the nanoscale electrode was comprised of nanofibers with embedded GOx. Both were placed between microfabricated electrodes and were principally comprised of a conducting polymer, poly(3,4-ethylenedioxythiophene) (PEDOT), and were tested at potentials of +0.3 V and +0.7 V<sup>30</sup>. As a result of its larger surface area, the nanofiber sensor exhibited an improved signal-to-noise ratio and a higher density of GOx immobilization than the bulk film. Additionally, the conductivity of the nanofiber sensor was greater than the bulk film system, and a 433% increase in sensitivity was reported<sup>30</sup>. Further, the detection limit for the nanofiber sensor was 0.26 mM, compared to 0.56 mM for the bulk polymer. The nanofiber sensor also exhibited significantly improved sensitivity over 30 days compared to bulk hydrogel (83% loss of sensitivity compared to 28% loss of sensitivity).

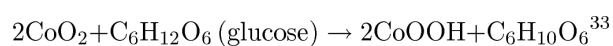
As a result of the superior surface area and reducing ability of nanostructures, highly sensitive glucose sensors can be fabricated with carbon nanotubes. Tang, et al. fabricated an amperometric sensor using multi-walled carbon nanotubes (MWCNTs) and Platinum nanoparticles (Pt NPs)<sup>27</sup>. The addition of Pt NPs to platinum electrodes containing chitosan-immobilized GOx reduced the glucose detection limit by a factor of greater than 1000 to an exceptionally low limit of detection of 5 nM as compared to a non-Pt NP functionalized sensor<sup>27</sup>. This drastic reduction in detection limit was attributed to the Pt NP's excellent ability to reduce hydrogen peroxide generated by the GOx reaction<sup>27</sup>.

To prevent contamination of the glucose signal by interfering chemical species, some groups have shielded their sensors from such species. This shielding is particularly important for sensors which utilize non-enzymatic glucose transducers, such as boronic acids. Zeng et al. immobilized GOx onto gold nanoparticle electrodes (AuNPs) with a chitosan hydrogel and covered the matrix with a thin Nafion layer to prevent interfering species from entering the sensor<sup>18</sup>. The sensor exhibited excellent performance as a result of the inclusion of AuNPs and despite the Nafion layer. The GOx-generated hydrogen peroxide was catalysed by the gold nanoparticle electrodes and the resulting current was measured<sup>18</sup>. The sensor was 2.8 times more sensitive to glucose than a sensor fabricated with a flat gold electrode, and a linear detection range of 3.0  $\mu$ M – 9.0 mM with a detection limit of 1.5  $\mu$ M and a response

time of < 5 seconds was reported<sup>18</sup>. Additionally, Uehara, et al. developed a sponge like nanoporous membrane with a pore diameter of 5–30 nm<sup>16</sup>. This membrane was used to selectively allow the passage of glucose, while blocking larger albumin molecules<sup>16</sup>. Applications of this membrane could include shielding a glucose sensor from interference from larger molecules or preventing aggregation of large molecules on its surface. Nunes and co-workers developed a membrane capable of serving the same purpose, but with pH responsive pore diameters; pore diameters from 4–17 nm could be achieved over a pH range of 4.2–7<sup>31</sup>.

The ability to detect both increasing and decreasing glucose concentrations, known as reversibility, is another important characteristic of glucose sensors and a requirement for an implantable continuous glucose sensor. Zhai and co-workers developed a highly reversible sensor with minimal hysteresis by immobilizing GOx and Platinum Nanoparticles (Pt NPs) within a polyaniline hydrogel matrix, and used the matrix as a conductor to measure the electrical activity of both the electrocatalytic action of the GOx enzymatic reaction and the Pt NP mediated catalysis of hydrogen peroxide<sup>32</sup>.

It is important to note that enzymes can be easily denatured, and enzymatic sensor lifetime may be an issue which precludes continuous *in vivo* glucose sensing<sup>22</sup>. Therefore, enzyme-free modalities have been developed. Dong, et al. reported growing Cobalt oxide nanowires on graphene foam, which are capable of direct detection of glucose (Figure 3)<sup>22</sup>. Ding, et al. found that the cobalt nanowires serve as a catalyst for facilitating the direct oxidation of glucose to gluconolactone *via* the following equation<sup>33</sup>:



High sensitivity (3.39 mA mM<sup>-1</sup> cm<sup>-2</sup>) with a low detection limit (<25 nM) was reported by Dong, et al. in glucose concentrations from 0–1 mM<sup>22</sup>. Additionally, this reaction was found to be specific to glucose, as the catalysis of uric and ascorbic acid was not catalysed by the nanowires<sup>22</sup>. Similarly, silicon nanoparticles can be used for direct enzyme-free detection of glucose<sup>20, 21</sup>. Wang and coworkers were among the first to report such a system, and immobilized Si<sub>29</sub> nanoparticles on a Si substrate<sup>21</sup>. The electrical activity of the catalytic reaction was effectively transported to the substrate, and a linear detection range from 0–50 mM was reported. The system did not exhibit interference from ascorbic or uric acids<sup>21</sup>.

Another exciting option for glucose detection is the magnetic response technique reported by Sun and co-workers, who functionalized magnetic nanoparticles with Con A<sup>34</sup>. They found that the T2 relaxation time of the Con A functionalized nanoparticles was affected by the concentration of glucose due to the change in total particle mass, and reported a linear relationship between T2 relaxation time and glucose concentration (0–300 mg dL<sup>-1</sup>)<sup>34</sup>. The sensor was reported to exhibit excellent stability and is reversible, thereby making such a sensor a good candidate for continuous glucose monitoring. Furthermore, the toxicity of Con A has also been recently assessed, and has been found to be safe at low concentrations<sup>35</sup>.

## Optical Glucose Measurements

Optical glucose measurements typically involve an increase in fluorescence intensity or a shift in the wavelength of the light produced by a fluorescent material coupled with a glucose sensitive material. The implantation of reversible optical sensors has led to the concept of a “smart tattoo” which continuously measures BGL by modulating the tattoo’s optical wavelength in response to glucose concentration<sup>36</sup>. Optical sensors have a number of advantages, including imperviousness to electromagnetic fields, capability to be miniaturized for single molecule detection schemes, and the ability to be used in continuous real time glucose monitoring<sup>11</sup>.

Carbon nanotubes exhibit properties which make them ideal for fluorescent biosensing, such as emission at a near-infrared wavelengths, which is a wavelength at which skin is particularly transparent<sup>23</sup>. Barone, et al. was among the first to use single walled nanotubes (SWNTs) for fluorescent glucose sensing, and demonstrated that SWNTs functionalized with  $\text{Fe}(\text{CN})_6^{3-}$  exhibit a change in fluorescence when the  $\text{H}_2\text{O}_2$  generated by GOx action reduces  $\text{Fe}(\text{CN})_6^{3-}$  on functionalized SWNTs. This sensor was found to be sensitive to physiologically relevant glucose concentrations (1 mM – 8 mM) and was fully reversible<sup>23</sup>. Inherent fluorescence of carbon nanotubes was further investigated by Yum and co-workers (Figure 3), who tested 30 Boronic acids with SWNTs to find combinations which yield accurate and reversible changes in fluorescence signals in response to glucose concentration<sup>29</sup>. As glucose binds to several boronic acid functionalized SWNTs, the fluorescence signal increased. Two Boronic acids were found which optimized the desired properties: 4-cyanophenylboronic and 4-chlorophenylboronic acid, and a reversible sensor was realized by measuring the frequency shift of the fluorescence or the “turn on” response<sup>29</sup>. The sensor was sensitive to physiologically relevant glucose concentrations, and reacted quickly to changes in glucose (~1 min)<sup>29</sup>.

Additionally, it has been demonstrated that Glucose Binding Protein (GBP) bound to SWNTs can be used to mechanically actuate the SWNTs upon glucose binding, and thereby cause a quenching of the SWNT’s fluorescence in proportion to glucose concentration<sup>28</sup>. Such a sensor has been demonstrated by Yoon, et al, who bound GBP to SWNTs *via* amine coupling to lysine groups on the enzyme<sup>28</sup>. The group demonstrated a 5–10% decrease in fluorescence intensity in the presence of 20–40 mM of glucose, and a linear response was found from 2.5–10 mM. The system was fully reversible and generated stable responses for >60 h<sup>28</sup>. McNicholas, et al. investigated the chemical changes which occur as a result of immobilizing GBPs on SWNTs<sup>26</sup>. They found that GBP denatures at a lower temperature (4–5 °C) when bound to SWNTs, and were only found to be stable below 36 °C. This consideration should be taken into account when preparing glucose sensors with bound enzymes, especially when considering *in vivo* implantation for long term glucose monitoring.

Several optical sensor approaches utilizing metal nanoparticles have been developed. First, He and co-workers reported developing a highly sensitive glucose sensor by immobilizing GOx on Ag/Au nanoshells *via* poly(L-histidine)<sup>6</sup>. In this case, the hydrogen peroxide produced by the GOx enzymatic action caused the Ag coating to dissolve and expose



increasingly large areas of Au on the surface of the nanoshells, thereby causing a red shift in surface plasmon resonance peaks<sup>6</sup>. This shift, sampled at a standard time interval, could detect glucose concentrations from 0.5  $\mu\text{M}$  – 0.02 mM. Additionally, Bahshi and co-workers developed two optical techniques for detecting glucose<sup>8</sup>. The first used Glucose Dehydrogenase (GDH) to catalyse the production of NADH in the presence of glucose. The NADH caused a fluorescence increase in functionalized quantum dots (Hops Yellow Core/Shell EviDots (CdSe/ZnS QDs), which was used to quantify glucose concentrations. Alternatively, the growth of Au NPs can result from the production of NADH by GDH. Specifically, when  $\text{AuCl}_4^-$  ions are present in solution, they can be reduced by the NADH produced by GDH action, and lead to the growth of AuNPs. This causes an increase in fluorescence in the visible spectral region (peaks near 530 nm) as the AuNPs grow. Sampling time for both systems was on the order of 20–50 min, and glucose concentrations tested were on the order of 0–2.5 mM<sup>8</sup>.

Nanomaterials can also be used as a catalyst to cause fluorescence of materials in the presence of hydrogen peroxide. This has been exploited by Song and co-workers who demonstrated that carboxyl modified planar graphene can catalyse the oxidation of 3,3,5,5-tetramethylbenzidine (TMB) to produce a blue color when reacted with the hydrogen peroxide produced by GOx activity<sup>37</sup>. However, the catalysis of TMB by the carboxyl-graphene requires a pH of 4, which denatures the GOx enzyme. A two-step process was used to overcome this, and a highly sensitive glucose detection range from 1  $\mu\text{M}$ –20  $\mu\text{M}$  was reported. Additionally, the glucose concentration of blood could be accurately measured.

Yetisen and co-workers (Figure 5) developed an optical glucose sensor by fabricating a poly(acrylamide) (pAAM) hydrogel functionalized with 3-(acrylamido)phenylboronic acid (3-PBA) and Ag NPs<sup>11</sup>. As glucose entered the hydrogel and bound to the PBA, the hydrophobicity of the hydrogel increased, causing it to swell in proportion to glucose concentration<sup>11</sup>. The swelling modulated the distance between the embedded AgNPs, and caused a glucose-dependent shift in the frequency of refracted laser light. After optimization, a possible wavelength shift of  $\sim 350$  nm across the visible spectrum was reported for glucose concentrations ranging from 0 mM–10 mM. Additionally, achieving a steady state response with this hydrogel took 50 min–1.5 h, but an analysis of the transient response was conducted, and by applying the results of this analysis the glucose concentration could be accurately estimated after only 5 min<sup>11</sup>. The sensor was reported to be more accurate than commercially available test strips, and could detect an exceptionally wide range of glucose concentrations (0.1 mM–375 mM)<sup>11</sup>. This sensor was designed to detect the glucose concentration of urine, which correlates with BGL<sup>11</sup>.

Veetil and co-workers developed a mutant glucose binding protein (GBP) which contains the fluorescence resonance energy transfer (FRET) probes AcGFP1 and mCherry<sup>9</sup>. Upon glucose binding, the FRET pair is brought in proximity to each other, causing a change in the FRET signal. When the FRET pairs are separated, the intensity of 505 nm light is significant, but decreases sharply upon glucose binding<sup>9</sup>. The sensor could accurately detect glucose in the range of 25–800  $\mu\text{M}$ <sup>9</sup>. Recently, this group reported expressing a similar mutant GBP protein within murine myoblast cells by transfecting the mutant GBP DNA into the cell's DNA<sup>15</sup>. The mutant GBP protein was synthesized within the cells, and could be

used to analyse the glucose concentration within the cells<sup>15</sup>. The resulting ability to see real time glucose metabolism within the cell is important for understanding diabetes pathology and may be useful in understanding possible intracellular therapies<sup>15</sup>. Furthermore, this approach may be especially useful for studying the pathology of type 2 diabetes<sup>15</sup>. Within the cell, elevated glucose concentrations were found close to the cell membrane, and low glucose concentrations were found close to the nucleus<sup>15</sup>.

A novel method for glucose detection developed by Stuart, et al. is the adaption of Surface Enhanced Raman Scattering (SERS) for *in vivo* glucose detection by coating the surface of Ag NPs with decanethiol and mercaptohexanol<sup>38</sup>. This facilitates the localization of glucose to the NP surface and leads to a detectable FRET signal based on glucose concentration<sup>38</sup>. Stuart, et al. demonstrated the system in a rat model, and found that the system was capable of detecting glucose concentration *in vivo* with accuracy comparable to a commercial glucose sensor<sup>12, 38</sup>.

## Nanotechnology Enabled Closed-Loop Insulin Delivery

An attractive alternative to traditional insulin injections are “closed-loop” systems, or systems which continuously monitor blood glucose and release insulin in a self-regulated fashion. There are several benefits of such a system over the traditional open loop system, including tighter control of BGLs, which can reduce the complications characteristic of diabetes<sup>39, 40</sup>. Closed-loop systems may also lead to a decrease in the dosage of insulin, and reduce the number of hypoglycemic and hyperglycemic events. These advantages have led to considerable interest in developing closed-loop systems. The reported systems have demonstrated favourable results in terms of responsiveness and biocompatibility<sup>7, 41</sup>.

## Microencapsulation of Islet Cells – Artificial Pancreas

Encapsulating the body’s own insulin producing  $\beta$ -cells within semi-permeable polymer matrices, such as alginate hydrogel, is an attractive option for closed-loop insulin delivery. This strategy calls for the encapsulation of  $\beta$ -cells within a semi-permeable membrane that shields the cells from host immune responses while allowing small molecules, nutrients, and insulin to passively diffuse across the membrane. Designing such a membrane is a complex challenge and is being approached from several perspectives. One of the most significant challenges to this approach is overcoming the host’s immune response to directly implanting foreign islet cells. Typically, the cells are rapidly engulfed by fibrotic overgrowths as a result of an immune response<sup>42</sup>.

Another technique for encapsulating islet cells is the Layer by Layer (LbL) deposition of a semi-permeable membrane onto the cells<sup>7, 43</sup>. A persistent challenge of using LbL deposition for islet cell encapsulation is avoiding the use of polyionic polymers, which are cytotoxic<sup>44, 45</sup>. Alternative binding strategies are needed to create LbL coatings on the cells that have increased stability and reduced cytotoxicity. Recently, Gattas-Asfura and co-workers reported a new LbL technique utilizing interpolymeric covalent linking to enhance the stability of the LbL coatings<sup>46</sup>. Alginate and poly(amidoamine) (PAMAM) dendrimers were functionalized with complementary Staudinger ligation groups: azide and methyl 2-(diphenylphosphino)terephthalate. Covalent linkages and electrostatic forces between the



films increased the stability and thickness of the resultant bilayer. This bioorthogonal functionalization technique for interpolymeric linking also serves as a method for functionalizing the surface of the islet and can be modified to include secondary therapeutic agents such as immunosuppressant drugs or bioactive groups that enhance the nutrient supply<sup>47</sup>. However, the coatings still face the issue of cytotoxicity due to the inclusion of polyionic polymers.

A solution to the polyionic induced cytotoxicity was recently reported by Kozlovskaya and coworkers<sup>41</sup>. The group reported an LbL coating strategy that used hydrogen bonding between tannic acid (TA), a natural polyphenol with poly(*N*-vinylpyrrolidone) (PVNOP) which holds the polymeric films together. The interactions between TA and the cell membrane proteins resulted in a highly stable coating. The hydrogen bonded films maintained good stability for up to 7 days after the initial coating. Furthermore, the cells coated with the bilayers displayed no significant increase in cytotoxicity compared to uncoated cells. Significant challenges in terms of immunoisolation of the islet cells remain before islet cell transplantation can become a clinically viable option.

### Glucose-Responsive Insulin Delivery

Synthetic closed-loop systems are often characterized by their method of glucose detection. Glucose can be detected through enzymatic methods using enzymes such as glucose oxidase (GOx), Binding of glucose to synthetic groups such as those in Phenylboronic acid (PBA), or binding to glucose binding proteins such as Concanavalin A (Con A)<sup>13</sup> as seen in Figure 6.

**Glucose Oxidase (GOx) Systems**—Glucose oxidase is capable of enzymatically catalysing the oxidation of glucose to gluconic acid with high specificity. The rate of enzymatic production of gluconic acid is directly related to the local glucose concentration. A typical closed-loop system utilizing glucose oxidase is comprised of a pH sensitive polymer matrix containing both insulin and GOx. The matrix undergoes a physical change such as hydrolysis or volume phase transition as the pH of the system is altered by gluconic acid production, thereby releasing the insulin entrapped within the matrix in a glucose dependent fashion<sup>6, 13</sup>.

Gu and co-workers recently developed a glucose-responsive nano-network for insulin delivery using GOx as the glucose sensing element (Figure 7)<sup>48</sup>. A double emulsion method was used to prepare acetal modified dextran (m-dextran) nanoparticles which included insulin, GOx and catalase (CAT). The nanoparticles were coated with either positively charged chitosan or negatively charged alginate. The opposing surface charges caused the nanoparticles to assemble into a porous 3D nano-network. The conversion of glucose to gluconic acid triggered the hydrolysis of the acetal groups to ethanol and acetone which results in the phase transition of hydrophobic m-dextran to dextran, which is hydrophilic. This phase transition results in the release of the entrapped insulin in a glucose-responsive fashion. The nano-network architecture endowed the system with sufficient mechanical strength to overcome a burst release at the injection site. Furthermore, the porous structure led to the presence of microchannels which facilitated higher rates of glucose diffusion through the nano-network leading to near zero order enzyme kinetics. The system showed

no significant insulin release at normal glucose levels ( $100 \text{ mg dL}^{-1}$ ), but transitioned to fast insulin release under hyperglycemic conditions ( $400 \text{ mg dL}^{-1}$ ). In *in vivo* studies, a single subcutaneous injection of the nano-network was capable of maintaining a diabetic mouse's blood glucose levels in the normoglycemic range for 10 days<sup>48</sup>.

More recently, Tai and co-workers developed an enzyme based biomimetic polymersome for glucose-responsive insulin delivery<sup>49</sup>. Polymersome nanovesicles were comprised of a Polyethylene glycol-poly (Ser-Ketal) PEG-Poly (Ser-Ketal) amphiphilic diblock polymer, GOx, CAT, and Insulin. Under neutral conditions the polymersome forms a bilayer structure which contains the insulin and allows glucose and oxygen to diffuse through the membrane. The enzymatic production of gluconic acid in response to hyperglycaemic glucose concentrations results in the degradation of the polymer membrane by degrading the ser-ketal linkage into PEG-Poly(Serine), ethanol, and acetone. The degradation of the membrane causes a subsequent release of insulin. Alternating the nanovesicles between hyperglycaemic conditions ( $400 \text{ mg dL}^{-1}$ ) and normoglycemic conditions ( $100 \text{ mg dL}^{-1}$ ) resulted in a pulsatile release of insulin with maximal release at the hyperglycaemic state, and negligible release under normoglycemic conditions. The polymersome bilayer structure led to excellent mechanical stability of the system. No insulin release was observed from the vesicles without a glucose stimulus over a course of 1 month. *In vivo* studies revealed that insulin released from the polymersomes retained bioactivity and were effective for regulating the glucose levels of diabetic mice for up to 5 days.

Gordijo and co-workers developed a glucose-responsive nanocomposite membrane for insulin delivery<sup>10, 50</sup>. The composite was prepared by conjugating  $\text{MnO}_2$  nanoparticles and poly(N-isopropylacrylamide-co-methacrylic acid) poly(NIPAM-MAA) with GOx, insulin, catalase (CAT), bovine serum albumin (BSA) and gluteraldehyde. The resultant PNIPAM-MAA membrane is pH sensitive and is capable of shrinking under the acidic conditions induced by hyperglycaemia. The shrinking of the membrane leads to insulin release across the membrane by increasing the porosity of the hydrogel network. However, degradation of the enzyme is a significant challenge for glucose-responsive systems utilizing GOx. As  $\text{H}_2\text{O}_2$  accumulates as a product of the enzymatic reaction, the activity of GOx is degraded. To address this, the CAT enzyme is often included to scavenge the  $\text{H}_2\text{O}_2$  and catalyse its conversion to  $\text{O}_2$ <sup>10, 48, 51</sup>. However, CAT is not capable of scavenging all the  $\text{H}_2\text{O}_2$ , and is itself affected by an increase in  $\text{H}_2\text{O}_2$  concentrations. The inclusion of  $\text{MnO}_2$  NPs works synergistically with CAT to scavenge more  $\text{H}_2\text{O}_2$ . The rigid  $\text{MnO}_2$  NPs increased the mechanical strength of the system as well the longevity of the enclosed enzyme. Inclusion of the  $\text{MnO}_2$  NPs and CAT led to a nanohybrid membrane that maintained excellent activity for 15 days, and maintained 65% of its initial activity after 30 days.

Most glucose-responsive insulin delivery systems utilize the degradation of a polymer matrix or a phase transition to mediate the release of insulin. However, attention has recently been directed to mesoporous silica nanoparticles (MSNs) as a result of their rigid structure and high surface area. Recently, Chen and co-workers developed a glucose dependent drug release system<sup>52</sup>. The system is comprised of MSNs loaded with rhodamine as a model drug. The MSNs are capped with GOx bound to N-glucosamine, a GOx inhibitor, which in turn is bound to the outside of the NP. As the glucose concentration increases, it competes

with glucosamine to bind to the GOx. Glucose binding to GOx results in uncapping of the MSNs and leads to the release of the encapsulated drug. *In vitro* studies indicated that rhodamine release from the NP was glucose dependent, and that no release was observed in the absence of glucose. Furthermore, drug release was correlated with glucose concentration. In addition to Rhodamine, other drugs such as Insulin could be stored within MSNs and capped with a glucose responsive element<sup>52–54</sup>.

**Glucose Binding Protein (GBP) Systems**—Glucose binding proteins can bind to glucose or glucosyl moieties in glycopolymers<sup>5</sup>. The most common class of glucose binding proteins are lectins. Lectins are a family of proteins that can bind to carbohydrates, and have a significant role in cell signaling<sup>55</sup>. The most common lectin used for insulin delivery systems is Concanavalin A (Con A). It is the preferred GBP because of its high affinity for glucose. Con A can bind to gluconic acid modified insulin (G-insulin) and be included in hydrogels. Under hyperglycaemic conditions, competition between G-insulin and glucose leads to the release of G-insulin in a glucose dependent manner. Wu and co-workers recently developed a MSN system capped with Con A<sup>56</sup>. The system is comprised of Con A bound to a mannose moiety, which is used to cap the pores of the MSN which contain rhodamine as a model drug. The resulting system was both pH and glucose-responsive. Triggering of the system by pH occurs because Con A exists as a dimer or monomer at low pH, and assumes a tetrameric state at neutral pH. Competitive binding of glucose with the capped Con A led to a release of the drug loaded within the MSN. In the absence of a glucose or a pH trigger, no drug release occurred.

**Phenylboronic Acid (PBA) Systems**—A disadvantage of protein based glucose sensing is that environmental factors such as pH and temperature must be tightly controlled to prevent protein denaturation. The possibility of denaturation limits the glucose sensing applications and sensor lifetimes of protein based systems. This poses challenges for the long term measurement of blood glucose by such sensors. An attractive alternative is the use of Phenylboronic acid and its derivatives as the glucose sensing moiety in both BGL measurement and insulin delivery applications. The ability of Phenylboronic acid to reversibly bind to polyols has been well established<sup>57</sup>. This has allowed Boronic acid derivatives to serve as the detection element in glucose-responsive insulin delivery and BGL measurement applications.

Phenylboronic acid in its neutral form exists as a trigonal planar hydrophobic molecule but is deprotonated under slightly basic conditions ( $pK_a > 8$ ) where it exists as a tetrahedral hydrophilic molecule with a net negative charge. The equilibrium between the two states is pushed towards the tetrahedral state in the presence of diols and polyols such as those found in glucose<sup>58</sup>. This equilibrium can be utilized to trigger the dissolution of amphiphilic nanocarriers and thus release insulin<sup>59–63</sup>. The equilibrium shift from the neutral to the charged form can also trigger the swelling of crosslinked hydrogel matrices.

Kataoka et al. were the first to report a synthetic glucose-responsive system for insulin delivery in 1998<sup>58</sup>. The group synthesized a polymer matrix of poly (*N*-isopropylacrylamide) (PINPAAm) that included PBA as a glucose sensing moiety. Upon glucose complexation with the PBA, the hydrophilicity of the matrix increased, causing the

matrix to swell and release insulin in a glucose-responsive fashion<sup>64</sup>. Since this initial work several other insulin delivery systems have been devised which utilize the PBA moiety for its glucose sensing properties<sup>48, 60, 65, 66</sup>.

One approach for glucose-responsive insulin delivery is the use of crosslinked polymer matrices with incorporated PBA. These matrices can reversibly swell when PBA forms a boronate ester with glucose. The ester formation leads to an accumulation of negative charge on the matrix, which results in repulsive forces that cause the cross-linked matrix to swell and release insulin. Recently, Wang and co-workers developed a co-polymer micelle comprised of poly(ethylene glycol)-block-poly-(acrylic acid-co-acryloamidophenylboronic acid) - (PEG-*b*-(PAA-co-PAAPBA))<sup>59</sup>. The micelle was capable of swelling in response to glucose concentration. The core of the micelle shifted from being hydrophobic to hydrophilic as the glucose formed a complex with the PBA moiety in the core of the micelle. The insulin stored within the micelle was then released in glucose dependent manner. In the absence of glucose the micelle was stable and no insulin release was detected.

One of the primary issues with using PBA in clinical systems is that the tetrahedral form requires a pH higher than those observed in most physiological systems. Yao and co-workers developed a polymeric glucose-responsive insulin delivery system that operates under neutral pH conditions<sup>60</sup>. Their system is comprised of an amphiphilic polymer made of Poly(ethylene glycol)-block-poly[(2-phenylboronic esters-1,3-dioxane-5-ethyl) methacrylate] (MPEG5000-block-PBDEMA). Electron donating groups were included within the polymer to increase its Lewis acidity and thereby reduce the pKa required to form the boronate ester. This co-polymer self assembles into micelles, and the system releases insulin upon the matrix's transition from amphiphilic to hydrophilic.

Yang et al. devised a similar system utilizing a PBA sensing scheme at physiological pH using micelles<sup>67</sup>. The micelles were prepared by synthesizing a block co-polymer containing poly(ethylene glycol)-*b*-poly(aspartic acid co-aspartamidophenylboronic acid) PEG-*b*-P(Asp-coAspPBA) along with a poly(Aspartic acid co aspartglucosamine)-P(Asp-co-AGA) glycopolymer. The introduction of glycopolymers into the polymer structure of the micelle allowed it to remain relatively stable under normoglycemic conditions (1 g L<sup>-1</sup>) due to the competition between the glycopolymer and glucose binding to PBA. However, at elevated glucose concentrations (2 g L<sup>-1</sup>), the micelles disintegrated and rapidly released insulin. The system was stable at normal blood sugar levels, and this stability reduced the risk of a burst release of insulin, which is highly desirable in a self-regulating insulin delivery device.

Polymer based insulin delivery systems have great versatility. Modifying the structure of the polymer can be used to tune the insulin release behaviour and sensitivity. Kim and co-workers synthesized a PEG-polyboroxole block co-polymer polymersome which was capable of self-assembly into micelles or cylindrical polymersomes based on the polyboroxole block length<sup>68</sup>. The group reported that at neutral pH, binding of the boroxole group to monosaccharides such as glucose (0.5 M) and fructose (0.2 M) was capable of producing charged boronate groups in the core of the polymersome. This change in charge modified the core's behavior from hydrophobic to hydrophilic, thereby causing the polymersome to dissociate and release embedded cargos such as insulin. The polymersomes

did not dissolve in the absence of monosaccharaides, and instead maintained their morphology for up to 3 months. The boroxole group showed a stronger binding affinity for fructose over glucose, but the versatility this system shows promise for self-regulated insulin delivery platforms.

Several groups have attempted to create platforms which exhibit a step wise response to glucose concentration and can deliver multiple drugs. Zhao and co-workers used Mesoporous Silica Nanoparticles (MSNs) as a platform for glucose-responsive release of Insulin and cyclic adenosine monophosphate (cAMP) – a trigger for the production of insulin from pancreatic beta cells<sup>69</sup>. The MSNs were loaded with cyclic AMP and capped with PBA bound to gluconic acid modified insulin (G-insulin). The system used the preferential binding of glucose to PBA to release the G-insulin-PBA cap from the MSNs, thereby liberating both insulin (in the G-insulin form) and cAMP. Furthermore, MSNs have been shown to be internalized by cells through endocytosis; this facilitates the uptake of cAMP, which normally encounters difficulty in crossing the cell membrane. The system was well realized and showed almost no leaching of cAMP without a glucose or fructose trigger. 20 mM glucose was capable of releasing 2  $\mu\text{M}$  of G-insulin at a MSN concentration of 2 mg mL<sup>-1</sup>. An ideal payload of insulin to lower blood glucose is about 200–350 pM<sup>69</sup>. Furthermore, the release of cAMP was also glucose-responsive at a glucose concentration of 100 mM; 60% of the loaded cAMP was released from the MSN. 0.25 to 0.3  $\mu\text{M}$  of MSN was capable of delivering a therapeutic dose of insulin in a glucose-responsive manner. The prospect of a dual delivery system is an exciting one, and has led to an increase in interest in MSNs for insulin delivery<sup>37, 52, 66, 70</sup>. However, the modification of insulin with gluconic acid raises questions about the bioavailability of the drug once released from the MSN.

Wu and coworkers designed a multifunctional hybrid nanogel system to serve as an insulin delivery device and glucose sensor (Figure 8)<sup>10, 62</sup>. The nanogel is comprised of poly(4-vinylphenylboronic acid-co-2-(diethylamino)ethyl acrylate) p[VPBA-DMAEA] polymer chains which serve as the glucose sensing moiety. The polymer is capable of crosslinking to form a nanogel which undergoes a phase transition in response to glucose concentration. The group included silver nanoparticles in the core of the nanogel that endows it with strong fluorescence. The wavelength of this fluorescence changes as the nanogel swells and shrinks in proportion to glucose concentration. In addition to the sensing mechanism, the system is also capable of dispensing insulin in a glucose-responsive fashion by swelling in response to hyperglycaemic conditions. The system was capable of phase transitions at a physiologically relevant pH (7.4) and exhibited high sensitivity to glucose; a lower detection limit of 0.75 mM was reported. The nanogels could release 56–91% of the stored insulin at high glucose levels, and could quickly modulate between high and low insulin release rates in response to changing glucose concentrations. Insulin release at basal levels could be sustained for over 2 days.

## Conclusion and Outlook

Significant advances in both glucose sensors and self-regulated insulin delivery systems have been facilitated by nanotechnology. Amperometric sensors utilizing nanotechnology now facilitate rapid, accurate, and highly sensitive glucose measurements in blood and other

clinically relevant fluids, such as tears and urine. Additionally, recent advances in fluorescent glucose detection holds the potential to lead to continuous *in vivo* glucose measurement. The realization of sensors which do not require repeated finger pricks to draw blood for glucose testing is highly desirable, as alternatives which avoid the pain, tissue damage, and patient noncompliance associated with the legacy clinical standard are highly desirable<sup>5</sup>. Furthermore, the clinical realization of a continuous glucose sensor could lead to closed-loop systems which could utilize existing insulin pumps<sup>6</sup>. This could relieve the patient of the significant burden of continuously managing their diabetes, and may tremendously improve their long term health outcomes and well-being. Based on current developments in nanoscale glucose sensing, we can expect great clinical applications of this technology in the near future<sup>71</sup>.

Progress in closed-loop insulin delivery systems has been encouraging and shows tremendous promise for the treatment of diabetes. Recently, a Con-A based polymer approach developed by Zion, et al has been close to clinical trials, following his company's 2010 acquisition by Merck<sup>72, 73</sup>. Current closed-loop systems are capable of releasing large amounts of insulin; however, clinical realization requires tight control of insulin release to reduce the risk of insulin overdose. Glucagon, a hormone which works antagonistically with insulin, could be co-delivered to reduce the risk of hypoglycaemia<sup>3</sup>. Additionally, because insulin is a growth factor, long term exposure to excess insulin can cause changes to the cell division process<sup>74</sup>.

Systems that offer remote control for the release of insulin are a promising development in dynamic insulin delivery. Stanley and co-workers developed nanoparticles coated with antibodies capable of binding to TRPV1 calcium channels on genetically engineered cells. The application of a 465 kHz RF signal causes localized heating of the channel *via* heating of the nanoparticles, and this heating causes subsequent passage of calcium into the cell, triggering the production of insulin within the cell<sup>75</sup>. The cells then release this insulin into the bloodstream<sup>75</sup>. Additionally, Di, et al. demonstrated insulin release *via* the bursting of nanocapsules by focused ultrasound (FUS)<sup>76</sup>. A nano-network<sup>48</sup> comprised of PLGA nanoparticles containing insulin was used to form an insulin reservoir, which could be selectively degraded by the application of focused ultrasound and lead to insulin release in response to ultrasound application<sup>76</sup>. These remotely triggered strategies may lead to novel insulin delivery modalities which can reduce the pain associated with repeatedly injecting insulin and increase patient compliance,<sup>5</sup> and may serve as an element of closed-loop glucose therapies. The best way of tackling the challenges ahead is by taking a multidisciplinary approach and incorporating knowledge of material science, physical chemistry, and pharmacology to develop more nuanced systems that are dynamic and stable.

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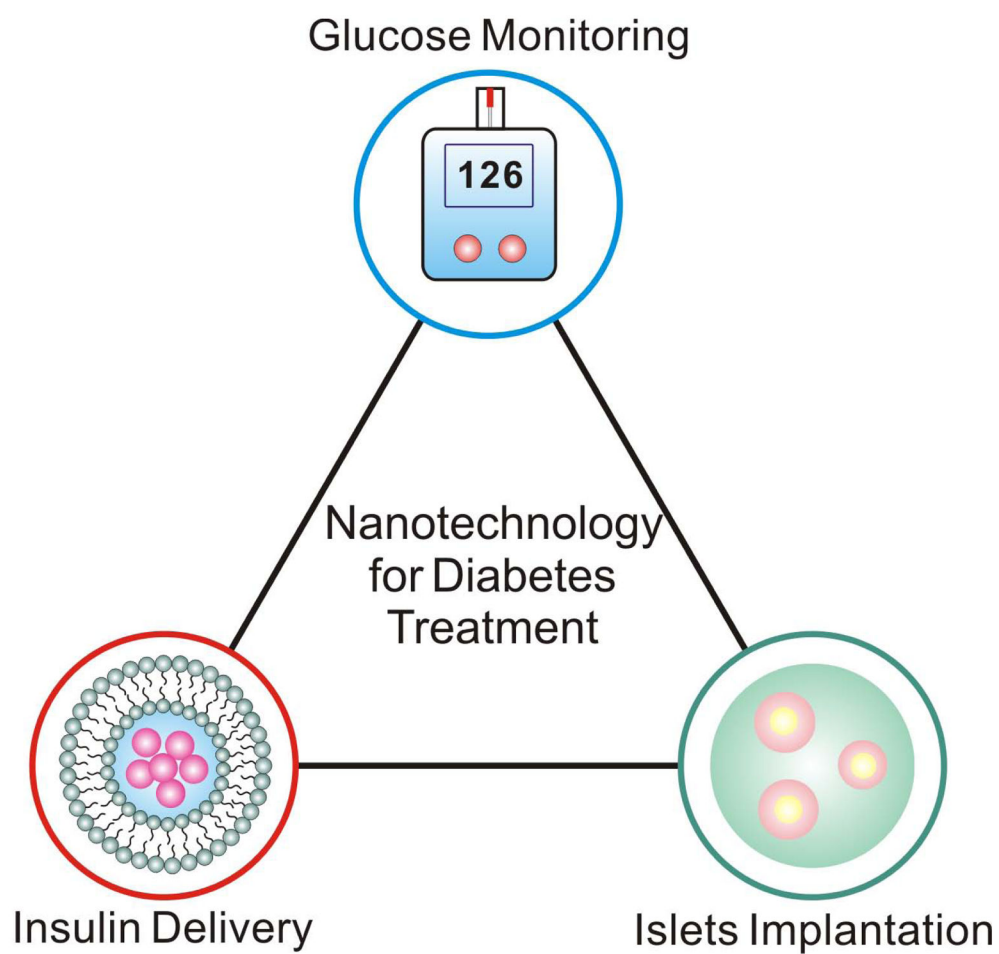
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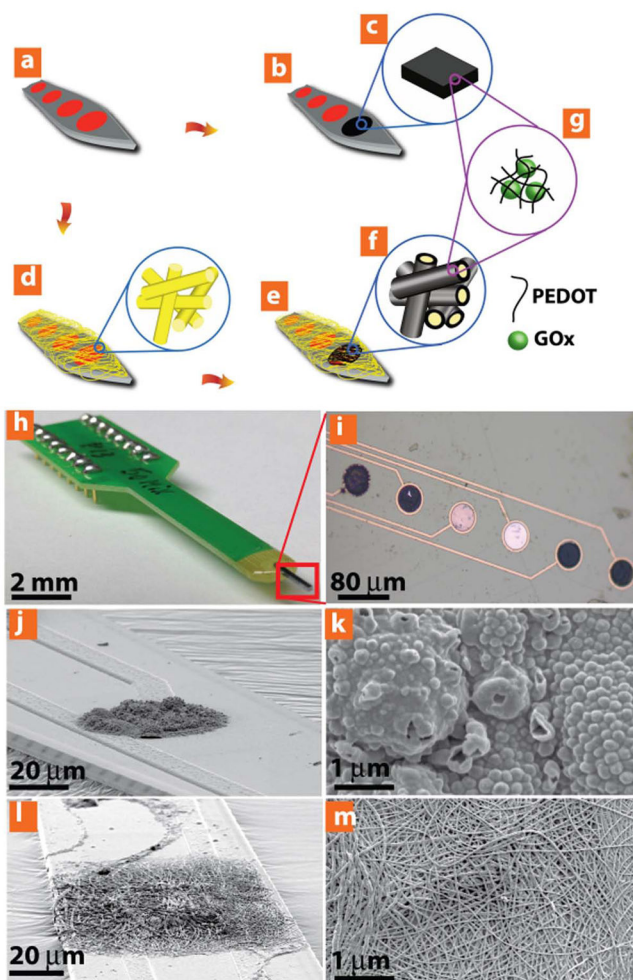
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**Figure 1.**  
Schematic of research themes using nanotechnology for diabetes treatment.

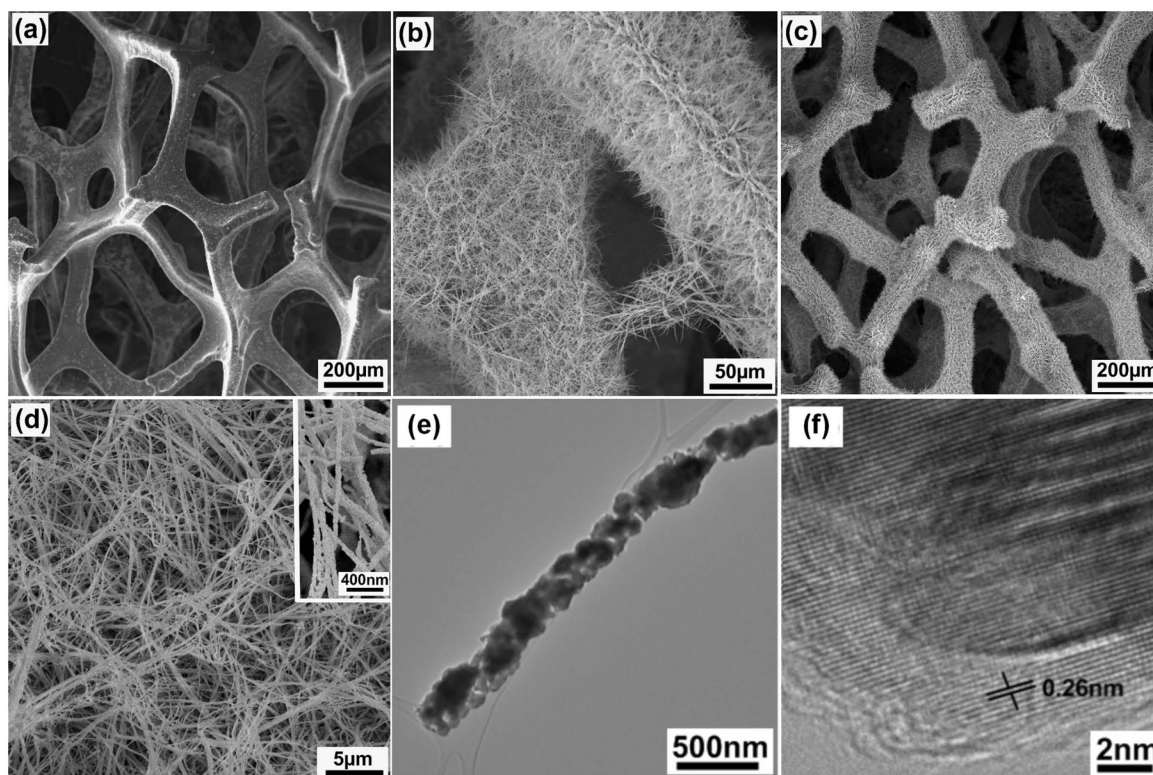




**Figure 2.**

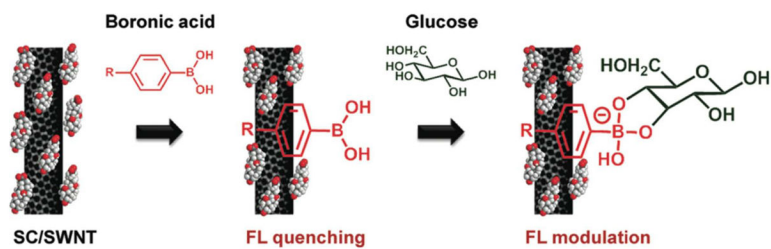
Yang, et al investigated how nanowires can affect the sensitivity of amperometric glucose sensors. In this experiment, two types of sensors were developed; the first was formed by immobilizing GOx within a bulk hydrogel, and the second was formed by immobilizing GOx on the surface of conductive nanowires. This figure depicts a schematic of the two types of glucose concentration sensors: (a–c) A bulk hydrogel glucose sensor with embedded GOx. (a,d–g) A nanowire glucose sensor with embedded GOx. (h,i) The glucose sensor apparatus. (j–k) The bulk hydrogel-GOx sensing system. (l–m) The nanowire-GOx based glucose sensing system. The nanowire based sensor was more sensitive than the bulk film sensor, which was attributed to its larger surface area, increased GOx loading capacity, and lower electrical resistance. (Reprinted with permission from Ref 30. Copyright 2014 John Wiley & Sons)





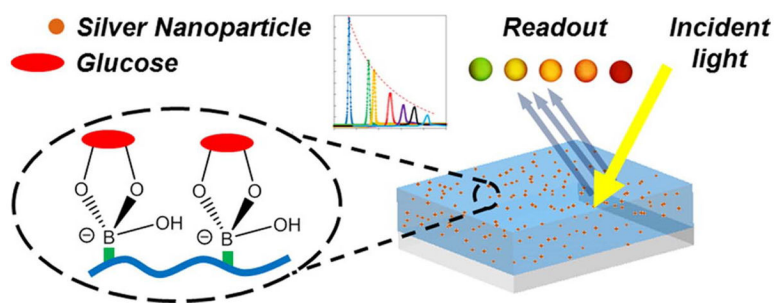
**Figure 3.**

The cobalt nanowires in this image can directly oxidize glucose without the use of enzymes, and thereby generate an amperometric signal proportional to the glucose concentration. An extremely small detection limit was reported ( $<25\text{nM}$ ), and was attributed to the extremely large surface area of the sensor. This figure depicts scanning electron microscopy images of (A) Graphene foam; (B) Cobalt nanowires grown on the surface of graphene foam; (C) Graphene Foam functionalized with cobalt nanowires; (D) High magnification of cobalt nanowires; (E) Image of a single cobalt nanowire. (Reprinted with permission from Ref 22. Copyright 2012 American Chemical Society)



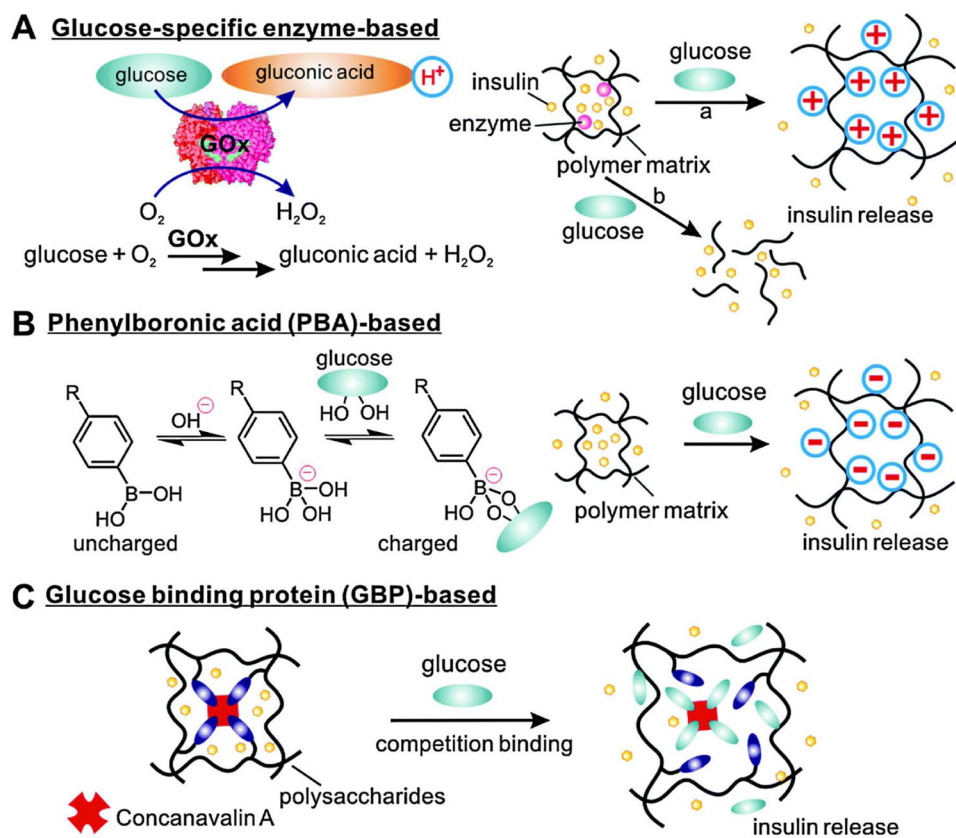
**Figure 4.**

In this study, Yum, et al used fluorescent carbon nanotubes with boronic acid to fabricate an optical glucose sensor. As the boronic acid binds to the nanotubes, the fluorescence is quenched. As glucose binds to these boronic acids, the fluorescence returns. Two Boronic acids were found which optimized the desired properties: 4-cyanophenylboronic and 4-chlorophenylboronic acid. (Reprinted with permission from Ref 29. Copyright 2011 American Chemical Society)

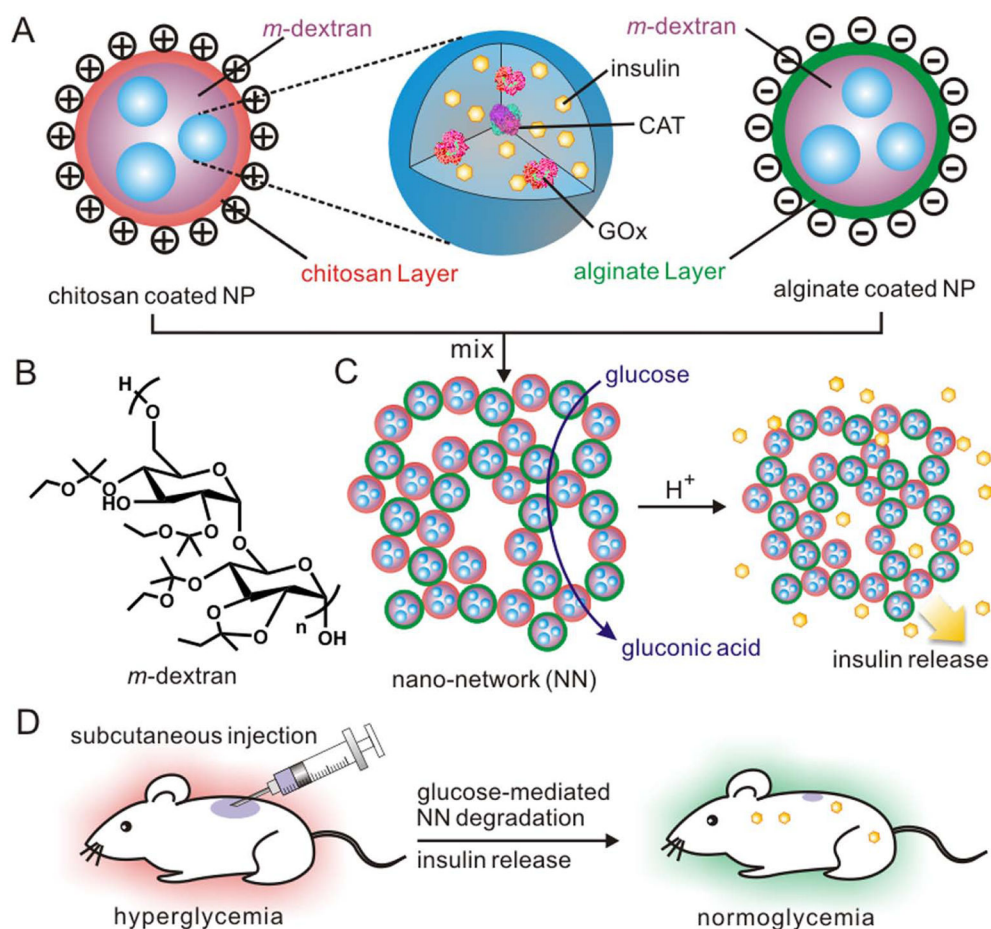


**Figure 5.**

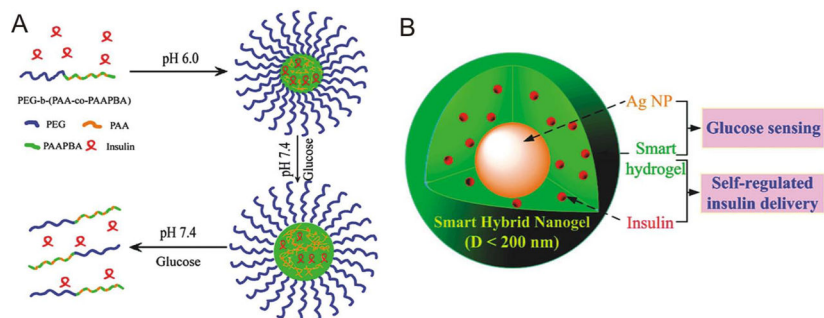
In this study, a boronic acid functionalized hydrogel was formed and included suspended silver nanoparticles. The functionalized hydrogel swelled and contracted in proportion to glucose concentration, as a result of the binding interaction between glucose and the boronic acid. As the hydrogel swells, the distance between the Au NPs modulates in response to glucose concentration, and thereby causes a shift in the wavelength of the refracted light. A wavelength shift of 350nm across the visible spectrum was reported for glucose concentrations ranging from 0mM–10mM. (Reprinted with permission from Ref 11. Copyright 2014 American Chemical Society)



**Figure 6.** Schematic of typical strategies for glucose responsive insulin release. (Reprinted with permission from Ref 5. Copyright 2014 Chemical Society Reviews)



**Figure 7.** Schematic of the glucose-responsive nano-network. (A) Acetal modified dextran nanoparticles containing insulin and GOx. The nanoparticles are coated with positively charged chitosan or negatively charged alginate. (B) Acetal modified dextran molecule. (C) Assembly of oppositely charged nanoparticles to form a nano-network and insulin release upon the enzymatic generation of gluconic acid under hyperglycaemic conditions. (D) Injection of the nano-network into a STZ-induced diabetic mouse model. (Reprinted with permission from Ref <sup>48</sup>. Copyright 2013 American Chemical Society)



**Figure 8.**

Glucose-responsive insulin delivery systems utilizing phenylboronic acid. (A) Insulin release *via* pH responsive micelles. (B) Multifunctional hydrogel for glucose-responsive insulin release and glucose concentration measurement. As the smart hydrogel swells in response to hyperglycemic conditions, the insulin is released and the fluorescence of the silver nanoparticles (AgNPs) is modulated. This fluorescence change can be detected and used to quantify the glucose concentration and the release of insulin. (Reprinted with permission from Ref <sup>62</sup>. Copyright 2012 American Chemical Society)



**Table 1**

Summary of reported glucose measurement systems.

| Type                          | Detection Principle                          | Response Time | Detection Limit               | Reference Number |
|-------------------------------|--|---------------|-------------------------------|------------------|
| Optical                       | Nanotube Near-IR emission                    | ~1 min        | 34.7 $\mu$ M                  | 23               |
|                               | Nanotube Fluorescence Enhancement            | ~1 min        | 2.5 mM                        | 28               |
|                               | Fluorescence Enhancement and AuNP Growth     | 30–60 min     | 0.01 mM (QDs); 0.1 mM (AuNPs) | 8                |
|                               | Graphene Catalytic Activity                  | 1h            | 1 $\mu$ M                     | 37               |
|                               | Nanotube Fluorescence Enhancement            | ~1 min        | 5 mM                          | 29               |
|                               | Raman spectroscopy                           | ~10 min       | 0.5 $\mu$ M                   | 6                |
|                               | Hydrogel Mediated Bragg Diffraction          | 5 min         | 90 $\mu$ M                    | 11               |
|                               | Protein FRET signal                          | ~1 min        | 25 $\mu$ M                    | 9                |
| Electrical                    | Nanotube conductance modulation              | ~20 s         | 0.1 mM                        | 24               |
|                               | Hydrogen Peroxide catalysis via Nanotubes    | <20 s         | 0.08 mM                       | 25               |
|                               | Hydrogen Peroxide catalysis                  | <5 s          | 1.5 $\mu$ M                   | 18               |
|                               | Hydrogen Peroxide catalysis via AuNPs        | <30 s         | 180 $\mu$ M                   | 17               |
|                               | Nanomaterial enhanced conductance modulation | <20 s         | 0.5 $\mu$ M; 5 $\mu$ M        | 27               |
|                               | Nanomaterial enhanced conductance modulation | <20 s         | 0.56 mM; 0.26 mM              | 30               |
|                               | Enzyme-free glucose catalysis                | <20 s         | <25 nm                        | 22               |
|                               | Nanoparticle catalysis of Hydrogen Peroxide  | 3 s           | 0.7 $\mu$ M                   | 32               |
| Enzyme-free glucose catalysis | <20 s  | 1 mM          | 21                            |                  |
| Magnetic                      | Shift in magnetic resonance of a membrane    | <1 min        | --                            | 34               |