Design and Development of Advanced Glucose Biosensors via Tuned Interactions between Marine Polysaccharides and Diagnostic Elements – A Survey

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Abstract

Extensive scientific analysis on the susceptibility of different populations to COVID-19 highlights that compared to populations with no co-morbidities, people with co-morbidities such as diabetes mellitus (DM) are at a significantly higher risk of serious infection, hospitalization, or even death. This underscores the importance of controlling DM through self-monitoring of blood glucose (SMBG) or continuous glucose monitoring (CGM) using biosensors. These biosensors, which can be either enzymatic or non-enzymatic, have undergone several rounds of development in terms of the materials used for device construction. In terms of the immobilization agent needed to anchor enzymatic or non-enzymatic detection elements to the electrode surface, marine polysaccharides, such as chitosan and alginate, hold a distinct advantage. This, in turn, can be ascribed to their biocompatibility, chemical stability, film-forming ability, capacity to bind with enzymes/diagnostic elements, and easy availability. In this review, we focus extensively on the use of cationic chitosan and anionic alginate in the past decade for designing advanced glucose biosensors. Their role in enhancing sensor response via physical/chemical interactions with the conducting and diagnostic elements is analyzed in detail from a structural point of view. In addition, the possibility of using these

polysaccharides in non-invasive CGM sensors is discussed and several potential future research avenues are presented.

Keywords: Biosensor, chitosan, alginate blood glucose, enzymatic/non-enzymatic sensors, sensor response, continuous glucose monitoring

1. Introduction

Over the past couple of years, the world has been ravaged by COVID-19, which has claimed more than 3.8 million lives at the time of writing this article [1]. People suffering from comorbidities have been inordinately susceptible to infection by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for COVID-19. One such common comorbidity that has resulted in increased hospitalization and mortality is *diabetes mellitus* (DM). A recent study reported that in China alone, in people affected by COVID-19, the mortality rate was 3 times higher for diabetic populations when compared to the general population [2]. Apart from COVID-19, DM increases the human body's susceptibility to other comorbidities and fatal scenarios. For instance, there exists a strong interplay between DM and cardiovascular disease (CVD), which has been identified to be a prevalent cause of morbidity and mortality in diabetic patients. In the United States, CVD death rates are 1.7 times higher among adults (>18 years) with DM when compared to adults with undiagnosed or no diabetes [3]. These numbers are of extreme concern considering that in 2019, 463 million adults (20–79 years) have been diagnosed with DM and this number is expected to climb steeply to 578 million by 2030 [4].

DM is an endocrine disorder resulting from a lack of adequate insulin, a peptide hormone responsible for glucose absorption in cells and the reduction of sugar levels in blood. Any failure in the mechanisms regulating insulin production leads to spikes in the blood sugar level [5]. Therefore, blood glucose is one of the most commonly used criteria to monitor DM, both in clinical and home-care settings, the latter of which is dependent on self-

monitoring of blood glucose (SMBG) and continuous glucose monitoring (CGM) using biosensors that can “read” blood sugar levels [6]. principle of enzymatic detection; more recently, non-enzymatic sensors have been introduced to improve the quality of glucose detection [7]. Thus far, two types of oxidoreductases, viz. glucose oxidase (GOx) and glucose dehydrogenases (GDHs), have been identified as efficient enzymatic elements for blood glucose detection. The underlying reaction schemes have been discussed in detail in many other notable reviews and hence, it would be redundant to described them here once again. Although these enzymes exhibit different interaction potentials with external oxygen acceptors, they are similar in terms of their reaction with the reducing end of glucose [8]. However, even though enzymatic sensors are considered to be highly accurate, they do face some drawbacks such as an extremely high dependence on enzymatic activity and enzyme stability with respect to ambient conditions. Therefore, research interest is gradually shifting toward non-enzymatic glucose biosensors, in which glucose is oxidized directly on the electrode surface via an electrochemical reaction, thus eliminating the need for costly and often unstable enzymes.

In a biosensor, these enzymes or non-enzymatic electrochemical agents, which act as recognition elements for target glucose molecules, are immobilized on a transducer, which converts the chemical output of the recognition reaction, into a readable signal [8]. The choice of the immobilization agent depends on several factors – biocompatibility, chemical stability, film-forming ability, capacity to bind with enzymes/diagnostic elements, and easy availability. Two systems that meet all the stated requirements for immobilization agents are the macromolecules chitosan and alginate, both of which are marine polysaccharides. These ubiquitously available materials, therefore, are used time and again in building enzymatic and non-enzymatic sensors for glucose monitoring. Because these systems assume high technological importance in the current global healthcare scenario, it is necessary to comprehensively analyze their application in glucose detection and monitoring and hence, the objective of this review is to systematically map the developments in chitosan and alginate-based glucose biosensors that occurred over the past decade. In addition, major research challenges and

opportunities, particularly related to non-invasive glucose monitoring have been highlighted.

2. Interplay between glucose sensors and the structural and chemical characteristics of chitosan and alginate

To understand the excellent application potential of chitosan and alginate in glucose biosensors, it is necessary to delve into their structural and chemical characteristics, which in fact dictate their behavior in different environments. The surface charge on these polysaccharides plays a key role in facilitating electron transfer between enzyme and the electrode surface, thus activating the catalytic function of the enzyme for rapid biosensing [9]. Therefore, in this section, the relevant structural and chemical properties of chitosan and alginate vis-à-vis glucose biosensors will be briefly discussed.

2.1 Chitosan

Chitosan, a linear polysaccharide derived from the sodium-hydroxide-based deacetylation of chitin [10], which in turn is extracted from the exoskeletons of crustaceans, some insects, and a few fungi, consists of N-acetyl-D-glucosamine and D-glucosamine units (Figure 1a). Industrially, chitin is extracted from shrimp and crab shells by acid treatment followed by alkaline treatment; subsequently, the obtained chitin is subjected to partial deacetylation to yield chitosan. The large number of oxygen- and nitrogen-containing active functional groups on the backbone of this polysaccharide hold the key to its strong application potential for various applications, including biosensors, as they allow multiple functionalization and derivatization paths. This feature will be discussed in detail in the following sections.

In contrast to chitin, which is insoluble in water due to its high degree of acetylation, the solubility of chitosan in aqueous media can be controlled by modulating environmental pH and ionic strength. At $\text{pH} < 6$, the amino groups in chitosan undergo protonation

resulting in a polycation well-dispersed in water; however, at higher pH values, these groups experience deprotonation, resulting in an acute phase separation in water (Figure 1b). A significant consequence of such tunable solubility is that chitosan hydrogel films can be easily prepared by solution casting, which comes in handy for enzyme immobilization during biosensor fabrication [11]. Furthermore, the polycation structure of chitosan is responsible for its antimicrobial activity, which is an essential requirement when designing implantable biomedical devices.

Fig. 1. Chemical structure of chitosan. Reproduced with permission from [12]. Copyright MDPI

2.2 Alginate

Unlike chitosan, alginate is an anionic linear polysaccharide and is derived mainly from brown algae, such as *Macrocystis pyrifera*, *Laminaria hyperborea*, *Laminaria digitata*, *Ascophyllum nodosum*, and to a small extent, from bacterial strains, such as *Azotobacter* and *Pseudomonas* [13, 14]. While the former involves treatment of alginic acid derived from dried and milled algal material with alkali solutions such as NaOH or Na_2CO_3 , the latter involves a series of biochemical pathways (synthesis of precursor substrate, polymerization and cytoplasmic membrane transfer, periplasmic transfer and modification, and export through the outer membrane) that result in the formation of alginate as an exopolysaccharide [13].

Structurally, alginate consists of blocks of consecutive or alternating 1,4-linked- β -D-mannuronic acid (M) and 1,4- α -L-guluronic acid (G) residues (Figure 2) and depending on the source material, the M/G composition as well as the block length vary. It has been posited that the specific stereoselectivity of the G and M units in this polysaccharide render this material a suitable substrate for highly selective reactions. As shown in the figure, the alginate chain contains a multitude of carboxylate and hydroxyl groups that can be manipulated for derivatization and enzyme immobilization. An oft-used method to produce alginate hydrogels is CaCl_2 -induced gelation, during which reaction, monovalent sodium ions in the carboxylate groups of M and G residues are replaced with divalent calcium ions to yield an *in situ* crosslinked “egg box” structure.

Fig. 2. Chemical structure of alginate showing chain conformation with consecutive (MM or GG) and alternating (MG) residues. Modified with permission from [14]. Copyright Hindawi

3. Chitosan-based biosensors for blood glucose monitoring

3.1 Enzymatic sensors

As described in Section 2.1, the structural features of chitosan, viz. the large number of hydroxyl and amino groups on its backbone, make it eminently suitable for derivatization with other components of a biosensor or for enzyme immobilization. Based on this principle, Şenel [15] developed an amperometric polypyrrole cross-linked chitosan/glucose oxidase/gold nanoparticle sensor (amperometric sensors measure

current response to detect analyte concentration at a fixed potential) for monitoring glucose levels in blood plasma; in this case, pyrrole-branched chitosan acted simultaneously as an immobilization agent for GOx and as a reagent to stabilize gold nanoparticles via electrostatic interactions. This highly sensitive biosensor exhibited a detection limit and sensitivity of 0.068 mM and 0.58 $\mu\text{A}/\text{mM}$, respectively. Meanwhile, Kumar Krishnan's group investigated the utility of chitosan-covered Pd@Pt core-shell nanocubes and chitosan-supported silver nanowires as electrochemical platforms for glucose detection [16, 17]. In the former case, Pd@Pt core-shell nanocubes were employed to enhance the electrocatalytic activity of the biosensor. Here, chitosan played a dual role – 1) it improved the biocatalytic activity of the nanostructures as a capping agent and 2) it immobilized the GOx enzyme on the treated nanocubes via covalent linkages. Similarly, in the latter scenario, chitosan induced the self-assembly of GOx enzymes through covalent coupling across its pendant -OH and $-\text{NH}_3^+$ groups and these were then immobilized on the surfaces of silver nanowires deposited on a glassy carbon electrode with glutaraldehyde as an external crosslinker. Thus, the electrical contact facilitated by chitosan between the metal nanoparticles (Pd@Pt or silver nanowires) and GOx improved direct electron transfer (DET) between the redox enzyme molecules and electrode surfaces, resulting in highly sensitive and stable glucose measurements.

In another amperometric biosensor for glucose detection, NiFe_2O_4 nanoparticles and GOx enzyme were dispersed in chitosan to form a chitosan/ NiFe_2O_4 /GOx system that could be coated on glassy carbon electrodes [18]; to realize a good amperometric response, ferrocene carboxylic acid was used as an electron-transfer mediator. The researchers found that at pH 7.0, this biosensor exhibited high sensitivity and long-term stability and ascribed this observation to the pKa of chitosan (~ 6.3). Below this value, chitosan becomes water soluble, resulting in free-floating enzymes and consequently, a low response.

To enhance enzyme immobilization and sensor specificity for glucose monitoring, it is possible to leverage coenzymatic reactions. Zhong et al. developed a pseudobienzymatic sensor for glucose monitoring using chitosan-Prussian blue-graphene composite

nanosheets, gold nanoparticles, and GOx [19]. Prussian blue, which is a strong reducing agent, played a significant role in reducing the effect of interferents in blood samples, such as ascorbic acid and uric acid. Meanwhile, concanavalin A (Con A), a lectin protein, along with chitosan, increased enzyme loading and stability in the biosensor via specific interactions with the mannose residues of GOx. Chitosan, in addition to immobilizing the enzyme through covalent linkages, acted as a stabilization agent for gold nanoparticles. Due to this unique architecture, the biosensor exhibited an excellent and stable response in the glucose concentration range of 2.5×10^{-5} to 3.2×10^{-3} M with a detection limit of 1.0×10^{-5} M with negligible interference from other analytes. Although this study does demonstrate the feasibility of coenzymatic sensors based on chitosan for glucose monitoring, this domain has remained largely unexplored over the past decade.

One particular material class often used in conjugation with chitosan for glucose biosensors is graphene and its derivatives; such predisposition may be attributed to their good electronic conductivity, large surface area, excellent mechanical and thermal properties, and the interaction between the positively charged amino groups of chitosan and negatively charged graphene oxide, which in turn results in stable dispersions [20]. To further improve the binding between chitosan and graphene oxide, covalent binding may be induced by activating graphene oxide. In one such study, graphene oxide was blended with chitosan and it was shown that the sensitivity of biosensors fabricated with this material (with GOx as the redox enzyme) was 8 times higher than that of biosensors fabricated with graphene oxide alone [21]. In this instance, graphene oxide sheets were activated using the ethyl(dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) chemistry, which led to the opening of epoxide rings in graphene oxide and a subsequent esterification reaction with the amino groups of chitosan. Meanwhile, Fang et al. used chitosan to reduce insulating graphene oxide to realize a highly conductive graphene-like material, which was then used in conjugation with gold nanoparticles (formed by the reduction of HAuCl_4 by chitosan) to fabricate GOx-immobilized electrodes for glucose biosensors [20]. In addition to a fast response time of 5 s, these sensors exhibited good reproducibility, anti-interference ability, and long-term stability.

All the amperometric biosensors discussed thus far involved the solution casting of chitosan dispersions on electrode surfaces for enzyme immobilization. However, this approach faces an inherent disadvantage as it is difficult to control film thickness during solution casting. Therefore, techniques such as electrodeposition, which facilitate control over film thickness, are being investigated in biosensor construction [22, 23]. In this context, chitosan is uniquely suited for electrodeposition due to its pH-dependent solubility. One particular biosensor for glucose detection was fabricated by the electrodeposition of a chitosan-GOx biocomposite on stainless steel needle electrodes decorated with Pt-Pb nanoparticles [24]. Although chitosan electrodeposition is usually carried out using water reduction, this process necessitates high voltage/current densities, under which conditions the enzymes entrapped between polymeric chains might experience denaturation. To overcome this problem, in this study, water was replaced with p-benzoquinone and this reagent acted as a proton-consumption agent, thus reducing the voltage density required for the electrodeposition of chitosan. In another study, ferrocene-grafted chitosan, GOx, and single-walled carbon nanotubes (SWCNTs) were electrodeposited on freestanding 3D graphene foam electrodes to develop highly sensitive biosensors for glucose detection (detection limit as low as 1.2 μM) in human blood serum [25]. Compared to flat 2D electrodes, these 3D biosensor systems provide greater surface area for enzyme immobilization and therefore, are more sensitive and stable.

In certain cases, real-time monitoring of blood glucose, i.e., CGM, is preferred over intermittent testing (SMBG) to instantly identify serious aberrations in glucose levels and prevent fatal outcomes. A key requirement of the implantable biosensors used for CGM is that they should be non-toxic and should not prompt an immunogenic response from the body. In this context, chitosan is an excellent material for in vivo biosensor construction due to its proven cytocompatibility and antimicrobial nature [26]. Based on this premise, Zhou et al. designed a top-down strategy to self-assemble chitosan and alginate layers on a polysulfone hollow fibrous membrane, which was then used as a coating on stainless steel electrodes on which GOx was layered by electrodeposition [27]. The porous structure of the outer membrane ensured good contact between the enzyme

and glucose molecules, while chitosan facilitated a gentle yet firm immobilization of GOx on the electrode. Interestingly, it was noted that the deposition of chitosan and alginate on the polysulfone membrane enhanced sensor response, probably by limiting the transmission of glucose and increasing the transmission efficiency of electrons from the polysulfone membrane to the working electrode. Figure 3 I illustrates the responses of these sensors to intraperitoneal glucose injection (1.2 mg/g) when implanted transdermally in Sprague-Dawley rats. The response trend was quite similar to the discrete readings obtained by sampling blood from rat tail veins, which demonstrates the potential of these biochemical systems as implantable glucose biosensors. However, it is to be noted that the authors reported current response for only 70 min and conducted analysis on non-diabetic mice. To validate the applicability of these systems for CGM, it is necessary to run tests for longer time periods on diabetic animal models.

Fig. 3. I(a) Transdermal implantation of chitosan-alginate coated GOx-based biosensor in Sprague-Dawley rats and (b) current response of the implanted sensor vs. glucose concentration measured by sampling tail vein blood at discrete intervals. Reproduced with permission from [27]. Copyright American Chemical Society. II(a) In vivo ocular impact of a carboxymethyl chitosan-functionalized graphene biosensor. Corneal surface of the experimental rabbit (a) before and (b) after wearing the biosensor for 24 h. Fluorescein sodium-stained corneal surface of the rabbit eye (c) before and (d) after wearing the biosensor for 24 h. Reproduced with permission from [28]. Copyright American Chemical Society.

In another study on implantable biosensors, as glucose concentration in tears is similar to that in blood, carboxymethyl chitosan-functionalized graphene-based biosensors were developed for intraocular CGM [28]. Here, carboxymethyl chitosan, which is more hydrophilic than chitosan, was used to modify graphite flakes via ball milling and subsequently, GOx was immobilized in this reagent via an amidation reaction between enzymatic amino groups and -COOH groups on carboxymethyl chitosan. Corneal contact electrodes fabricated by solution casting of the enzyme-immobilized carboxymethyl chitosan-graphene biocomposite were then implanted in the eyes of New Zealand rabbits and glucose concentration in the secreted tears was monitored. One of the main takeaways of this study was that these biosensors, which exhibited a high sensitivity at $9.7 \mu\text{A mM}^{-1} \text{cm}^{-2}$, a broad linear range at 12 mM, and a good detection limit of 9.5 μM , could be worn for 24 h at a time without any adverse impact on the corneal structure or intraocular pressure (Figure 3 II). This study underscores the point that continuous glucose detection can be achieved using periodically removable implants and thus there is a strong possibility of non-invasive CGM biosensor development in the near future.

Because dietary habits play a large role in diabetes progression, there is growing interest in monitoring and controlling sugar levels in processed foods. For example, Artigues et al. designed a biosensor based on GOx immobilized in chitosan and highly ordered titanium dioxide nanotube arrays for quality control during food manufacturing [29]. In this sensor design, chitosan served as an entrapment agent to immobilize GOx on the

surfaces of conductive TiO_2 nanotubes. This biosensor, which exhibited a sensitivity of $5.46 \mu\text{A}\cdot\text{mM}^{-1}$, is to be particularly noted for its accuracy and robustness. Glucose concentrations measured by high performance liquid chromatography (HPLC) and the GOx/chitosan/ TiO_2 nanoarray biosensor in different food and drink items, such as soft drinks, dairy products, soy sauce, and tomato sauce, were found to be quite similar (relative standard deviation < 10%). Therefore, this sensor design offers an inexpensive and efficient alternative to costly methods such as HPLC. Chitosan-based glucose biosensors have also been built in conjugation with multi-walled carbon nanotubes (MWCNTs) and glutaraldehyde to test the freshness of meat products, such as beef, in terms of food browning [30].

While a large number of electrochemical sensors used for glucose detection are amperometric, biosensors that work on the principle of impedance detection are also often encountered. Impedance response, which can be obtained by dividing the AC potential applied by the AC current generated at the working electrode due to the immobilization of bioactive layers, is usually monitored by electrochemical impedance spectroscopy (EIS) [31]. This technique is particularly advantageous as it can be used to quickly, accurately, and precisely gauge the effect of electrode/interface modification on the final sensor response. Asrami et al. fabricated an impedimetric biosensor for glucose detection using CuO nanoparticles (nano CuO) as the transducer and chitosan to immobilize GOx via glutaraldehyde (Figure 4) [32]. In this system, nano CuO was sputter coated on a fullerene tin oxide (FTO) substrate and subsequently, chitosan-GOx solution activated with glutaraldehyde was coated on the FTO/nanoCuO system. One of the most important features of this sensor was that its response time was shorter than 4 s and it exhibited a high level of enzymatic activity for a time period longer than four weeks when stored at 4 °C; such excellent activity can be attributed to a high enzyme loading due to strong chitosan-induced immobilization.

Fig. 4. Schematic of the FTO/nano CuO/chitosan/GOx impedimetric sensor used for glucose detection in blood serum. Reproduced with permission from [32]. Copyright Elsevier.

Another method to fabricate impedimetric biosensors for glucose detection involves the use of sacrificial polymers. To illustrate this method, we refer to a recent study in which polyvinyl alcohol (PVA) was sacrificed to develop 3D hierarchical nanoporous ZnO/chitosan/GOx biosensors [33]. Using this technique, a PVA-nano ZnO hybrid film was initially formed on an FTO substrate, which was then subjected to annealing at 430 °C, a temperature much higher than the decomposition temperature of PVA. This resulted in a nanoporous ZnO structure, with a large surface area, onto which chitosan and GOx were immobilized. The authors of this study reported that the disparity between the isoelectric points of chitosan and GOx (9.0 and 4.2, respectively) resulted in a strong enzyme-immobilization effect. In the normal blood-glucose range of 4.4 to 6.6 mM, the readings generated by this impedimetric sensor were quite similar to those generated by spectrophotometry (mean relative standard deviation of 3.67%), which validates the application potential of these systems as glucose biosensors. This approach is unique in the sense that it can be extended to immobilize enzymes on polymeric layers with different isoelectric points.

An important point that should be highlighted here is that in contrast to amperometric sensors, which have been built using different transducing elements such as Pd/Pt electrodes, CNTs, and graphene, impedimetric sensors based on chitosan for glucose detection have used only metal oxide nanoparticles. However, there are reports of impedimetric sensors containing graphene and gold nanoparticles for semicarbazide or DNA detection. Therefore, in future, a broader material spectrum should be investigated in conjunction with chitosan to improve the limit of detection and sensitivity of impedimetric biosensors for glucose detection. Furthermore, there is little information on the ability of these sensors to filter out the effects of common interferents, such as urea and ascorbic acid, in blood serum and this topic merits further investigation.

3.2 Non-enzymatic biosensors

Even though the lion's share of the glucose biosensor market is taken up by enzymatic sensors, research and commercial interest is gradually veering toward non-enzymatic constructs. This is primarily due to the inherent drawbacks of enzymatic sensors, such as stability issues and stringent requirements for storage conditions. The performance of non-enzymatic glucose biosensors depends on the electrocatalysts included in the device structure toward glucose oxidation in response to an applied potential. In several such systems, chitosan has been used to enhance the overall catalytic activity and this functionality of chitosan will be analyzed in this section.

Chitosan has been employed to convert graphene to reduced graphene oxide (RGO), which was subsequently used to modify the surfaces of glassy carbon electrodes in a non-enzymatic sensor setup [34]. While graphene provides an excellent medium for the immobilization of catalytic elements, such as Cu-Co nanoparticles, it is insulating in nature, which results in a reduced device sensitivity. However, in suitable conditions, the large number of amino groups in chitosan can interact with the hydroxyl and carboxylic groups of graphene according to a redox mechanism to yield conducting RGO [20, 35], which can then be used as an electrode modifier. The resultant amperometric Cu-Co nanoparticle/RGO-chitosan/glassy carbon electrode biosensor exhibited a linear current

response in the glucose-concentration range of 0.015–6.95 mM with a sensitivity of 1921 $\mu\text{A cm}^{-2} \text{mM}^{-1}$ and a detection limit of 10 μM . Due to its film-forming ability, chitosan can also be used to immobilize catalyst and conducting agents on electrode surfaces, akin to its capacity to immobilize enzymes on electrodes. In one particular study, Au nanoparticles were dispersed in a chitosan solution to form conducting films on glassy carbon electrodes [36]. The immobilization of Pt nanoparticle catalysts on these porous chitosan-Au films resulted in an excellent contact between the electrocatalyst and glucose, while the conductivity of the modified chitosan film resulted in a good sensor response. Khalaf et al. used chitosan as a sacrificial carbon source to produce an N/S-doped carbon-silver nanoparticle complex, which was then used as a catalytic and conducting element on a glassy carbon electrode to construct non-enzymatic glucose biosensors [37]. Here, the role of chitosan was to form a conductive carbonaceous umbrella around the catalyst Ag nanoparticles and thus improve the detection limit of the sensor. Interestingly, the doping of chitosan with nitrogen and sulfur prior to thermal treatment improved the selectivity of the sensor toward common interferents in blood serum, such as urea, ascorbic acid, and sucrose.

Ice templating is another technique that has been used to take advantage of chitosan's beneficial features in non-enzymatic glucose biosensors [38]. Using this method, chitosan and CNTs were subjected to self-assembly induced by ice segregation to yield flexible 3D porous cryo aerogels with snubby CNT tips prominently arrayed on soft chitosan sheets. In addition, these aerogels provided highly suitable matrices for immobilizing electrocatalysts, such as $\text{Cu}_2\text{O}/\text{CuO}$ core/shell nanoparticles, which promoted the oxidation of glucose. Biosensors built with glassy carbon electrodes modified with these $\text{Cu}_2\text{O}/\text{CuO}$ chitosan/carbon nanotube films delivered excellent specificity to glucose over a glucose-concentration range of 0 to 40 mM. Such a broad detection range can, in part, be attributed to the high electrical conductivity of the CNT-modified chitosan nanosheet. The cryogel approach was also used to fabricate non-enzymatic glucose biosensors based on Au nanoparticles/MWCNTs/chitosan [39]. In this instance, Au nanoparticle-decorated MWCNTs were dispersed in a chitosan solution, which was subsequently deposited on a gold electrode; the entire electrode assembly was subjected to

cryofreezing and thawing to produce a non-enzymatic system for glucose detection. The polymeric cryogel formed on the electrode provided a large surface area for specific interactions between the catalyst Au nanoparticles and the analyte (glucose) while the MWCNTs facilitated electron transfer to the electrode. Furthermore, this cryogel was found to be very efficient at preventing catalyst detachment from the electrode surface, as evidenced by the long-term stability and reusability of the fabricated biosensor. Therefore, this system has been proposed as a flow-injection amperometric sensor that can be extended to analytes other than glucose as well.

While non-enzymatic chitosan-based glucose biosensors do overcome the disadvantages faced by their enzymatic counterparts, viz. high costs, enzyme instability, and stringent use conditions, thus far, to the best of our knowledge, there have been no reports on their use as implantable CGM sensors. This particular deficiency can be attributed to cytotoxicity that can possibly be induced by the relatively large amounts of catalyst loadings required in these systems. Therefore, we are of the opinion that this research gap provides a fertile area for future research on these otherwise promising biosensor systems.

4. Alginate-based biosensors for blood glucose monitoring

Alginate, the second marine polysaccharide considered in this review, is polyanionic in nature and contains a large number of carboxylate and hydroxyl functional groups that can be derivatized for application in biomedical devices. Owing to their biocompatibility, easy availability, stability, capacity for functionalization, and film-forming ability, alginates can be used to design both enzymatic and non-enzymatic sensors for blood-glucose detection. Nevertheless, their poor electronic conductivity necessitates the inclusion of other components in the electrode structure. Some illustrative examples of such biosensor devices are described in this section.

4.1 Enzymatic sensors

Márquez et al. designed an alginate-based amperometric sensor for glucose analysis in whole blood samples using a bienzymatic GOx and horseradish peroxidase system [40]. Ca^{2+} -crosslinked alginate hydrogel films, in which the enzymes were immobilized, were uniformly deposited on screen-printed gold electrodes, which acted as good transducers. One of the most advantageous features about this system is the ability to prepare the electrodes at normal conditions of room temperature (usually 25 °C and pH 7), which decreases the fabrication cost. Secondly, the authors reported that when the electrode surface was wiped with phosphate buffer saline (PBS) after analyzing a blood sample, the hydrogel films could be completely detached, leaving behind a fresh and reusable electrode. The alginate hydrogel structure provided a protective layer around the enzymes, preventing their detachment/slipping off from the electrode surface and inhibited biofouling due to environmental contaminants. Biosensors constructed from these electrodes could accurately discriminate between healthy and hyperglycemic samples, with a good sensitivity ($-0.27 \mu\text{A cm}^{-2} \text{mM}^{-1}$) and low limit of detection (126 μM) over a large linear range (2–12 mM). Efforts were also made to integrate enzymes immobilized in alginate microbeads in microfluidic systems to detect glucose in blood serum samples [41]. Because the permanent coating of microfluidic channels with alginate films containing enzymes would preclude the reusability of the sensor, a new approach was developed to facilitate the removal of GOx-containing alginate beads by incorporating a magnetic component in them. Thus, when an external magnetic force was applied, these beads could roll off and a new set of sensing beads could be “immobilized” in the microchannels (Figure 5). In this microfluidic device, an electrolyte-insulator-semiconductor (EIS) sensor was used as the electrode and the reaction between GOx and the analyte released hydrogen ions into the buffer solution, which in turn affected the surface potential of the sensor. Changes in the flat-band voltage (V_{FB}) were found to accurately represent variations in the glucose concentration of the tested blood serum samples. An important feature of this device is that the multiple channels present in the microfluidic device make it possible to immobilize alginate beads containing different redox enzymes, which in turn facilitates the sequential analysis of different biomarkers in the same sample.

Fig. 5. Enzyme-carrying alginate microbeads are immobilized on an EIS sensor by means of step-like obstacle and an external magnetic field. Sample is injected into the microchannel and reacts with GOx contained in alginate beads. Changes in the potential of the sensor surface during the reaction process are measured and used to determine glucose concentration. Reproduced with permission from [41]. Copyright Elsevier.

In a recent study, paper-based sensors were developed to monitor glucose concentrations in fruits using the principle of GOx-mediated alginate gelation [42]. The authors exploited the fact that alginate gelation with bivalent cations, such as Ca^{2+} , is dependent on the environmental pH. When an alginate solution containing CaCO_3 and GOx comes into contact with glucose, the latter undergoes oxidation to release gluconic acid and H_2O_2 . Beyond a particular acid concentration, the pH of the solution reduces to a level suitable for alginate crosslinking, which increases the viscosity of the hydrogel. By monitoring the diffusion diameter of these crosslinked solutions on microporous membranes, the viscosity of the hydrogel and consequently, glucose concentration in the test samples could be calculated in the range of 1.4–7.0 mM.

Using a more traditional approach, an alginate-GOx-CuO nanocomposite crosslinked with glutaraldehyde was coated on a platinum electrode and this system was used to construct biosensors for glucose detection. At the optimal conditions, these biosensors exhibited two linear ranges of 0.04 to 3 mM and 4 to 35 mM with different sensitivity values. Additionally, it could retain 78% of its activity after 15 days, which indicates good long-term stability [43].

An amperometric glucose sensor based on carbon nanospheres and GOx immobilized in sodium alginate was described by Han et al. [44]. Unlike most of the alginate-based systems discussed thus far, this biosensor used sodium alginate, which was crosslinked using glutaraldehyde. Composite films of sodium alginate containing carbon nanospheres and GOx were deposited on Pt electrodes by solution casting, which were then used as working electrodes in three-electrode cells. At an optimal solution pH of 6.5, the fabricated biosensor exhibited a reproducible and linear response in the glucose-concentration range of 10^{-6} to 4×10^{-3} μM , which the authors attributed to the excellent electronic conductivity of CNS and the diffusion ability of GOx in the sodium alginate film.

McShane and Srivastava's group worked extensively on developing minimally invasive "smart tattoo" alginate-based implantable biosensors for CGM [45–47]. To develop these intradermal sensors, which measured glucose content in the interstitial fluid (ISF), the researchers devised multilayer thin-film-coated dissolved-core alginate-templated microsphere sensors embedded with fluorescent dye-labeled enzymes, viz. fluorescein isothiocyanate carboxymethyl (FITC)-dextran and tetramethylrhodamine isothiocyanate (TRITC)-apo-GOx, to induce fluorescence resonance energy transfer (FRET). When these alginate microspheres come into contact with glucose, FITC-dextran is displaced due to the preferential binding between glucose and TRITC-apo-GOx (apo-GOx is an inactive form of GOx and does not catalyze glucose oxidation; however, it retains its glucose-binding affinity), which leads to variations in the relative emission intensity of FITC and TRITC. This, in turn, can be correlated with ISF glucose concentration. The key aspect to be noted here is the preparation of "dissolved-core" alginate microspheres. Initially, alginate microspheres containing the biosensing element were prepared by a co-emulsification technique and polyelectrolyte layers were deposited on these microspheres in a layer-by-layer manner. However, the problem with this system is that the dense crosslinked alginate layer restricts the diffusion of macromolecular dextran, which means that changes in the fluorescence intensity would be minimal and do not represent glucose concentration. To overcome this obstacle, the researchers proposed a partial de-crosslinking method, in which the core of the alginate microspheres was dissolved by reaction with sodium citrate-tris(hydroxymethyl)aminomethane

hydrochloride; meanwhile, the polyelectrolyte layers do not dissolve and thus the enzyme particles partially encapsulated in the dissolved-core alginate microspheres can easily diffuse to bind with glucose in the ambient ISF. In vitro cytotoxicity studies on L929 mouse fibroblast cells showed that these biosensing systems exhibited almost 100% cell viability, which implies that they have strong potential for application as implantable CGM sensors. There have also been reports on other optical sensing modalities, such as Pt-porphyrin, coencapsulated with GOx in alginate microspheres for glucose detection in the near infrared range [48].

Co-encapsulation of glucose-oxidizing enzymes, such as GOx with metal nanoparticles, in alginate microspheres also affords an easy UV-Vis spectrophotometric methodology to track glucose concentration in different media. For example, GOx and HAuCl_4 were coencapsulated in alginate microspheres and when these entities came into contact with glucose, GOx participated in a redox reaction, resulting in the release of H_2O_2 ; the released H_2O_2 acted as a reducing agent for AuCl_4^- ions, leading to Au nanoparticle formation. Depending on the size of these nanoparticles, the absorption intensity of the microspheres at 520–540 nm varied and it was found to represent glucose concentration [49].

Surprisingly, an extensive literature survey revealed no studies in the past decade on non-enzymatic sensors based on alginate for glucose detection. While it may be tempting to believe that this lack of information points toward an incompatibility between alginate and non-enzymatic biosensing elements, this supposition is not true as there are a number of reports on alginate and non-enzymatic systems for monitoring other biomarkers like urea [50], ascorbic acid [51], and H_2O_2 [52]. For example, as demonstrated in Section 3.2, RGO is a felicitous material for non-enzymatic sensors based on chitosan and these characteristics may be combined with alginate as well without the need for complex systems (owing to the functionalization potential of alginate). Therefore, this unexplored area holds a lot of promise for developing efficient SMBG and CGM devices for diabetes management.

5. Concluding remarks

In summary, a critical analysis of recent research on biosensors for glucose detection shows that marine polysaccharides, such as chitosan and alginate, can significantly aid the design and development of advanced systems, primarily due to their biocompatibility, chemical stability, film-forming ability, capacity to bind with enzymes/diagnostic elements, and easy availability. Chitosan, in particular, has been used extensively with graphene oxide and its derivatives, in constructing enzymatic sensors, due to its ability to immobilize enzymes on the electrode surface, and reduce insulating graphene oxide to RGO, which is electrically conducting. Therefore, it is evident that cationic polysaccharides, such as chitosan, can be used to improve electron transport across the electrode surface in enzymatic glucose biosensors, which is critical to enhance their performance. Similarly, in non-enzymatic sensors, chitosan has been used in conjunction with a variety of conducting materials to enable SMBG. However, there still remain several challenges that require rigorous analysis. Firstly, even though there is ample evidence available on the suitability of chitosan and alginate as immobilizing agents for glucose-detection elements, there seems to be little information on the combined use of these materials. Secondly, coenzymatic immobilization and detection is a lucrative topic that merits further investigation. The possibility of switching off one enzyme while using another on the same substrate to detect different biomarkers is an intriguing problem that can potentially be addressed by manipulating the charge characteristics of chitosan and alginate. A third issue is that solution casting seems to be the predominantly favored approach for immobilizing detection elements. However, as described in the article, this approach does not lend itself to films of controlled thickness; therefore, techniques such as electrodeposition should be employed in a scalable manner. Finally, it has been demonstrated that non-invasive CGM is possible with chitosan-based enzymatic glucose biosensors, which opens up the possibility of realizing continuous blood glucose trends without spiked readings, an artefact of discontinuous monitoring. Nevertheless, there are no reports of non-enzymatic glucose biosensors in this rich domain. Similarly, though alginate-based non-enzymatic biosensors are being employed for detection of biomarkers such as ascorbic acid and urea from blood and serum, we could find no reports on their use for glucose detection. Thus, we are of the opinion that by addressing

this research gap, it would be possible to develop efficient, inexpensive, and non-invasive biosensors for continuous glucose monitoring.

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