A 'Nano' Era for Blood Glucose Sensing

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Diabetes is a condition marked by the inability of the body to properly manage the level of glucose (sugar) in the blood. In Type 1 diabetes, the body does not make insulin, the hormone that regulates the usage of sugar in a human body. Individuals with Type 1 diabetes require the correct amount of insulin to keep blood sugar levels even before and after meals and other times. People with the disorder must therefore monitor their glucose levels regularly, usually by pricking their fingers several times in a day to collect blood and using a glucose monitor with test strips to detect blood sugar levels.

Without strict control of glucose, diabetic individuals suffer from serious and chronic complications, such as blindness and tissue damage. Roughly 5 to 10 % of people with diabetes are Type 1, which used to be known as juvenile-onset diabetes.

Biosensor technology, under such circumstances, has emerged as a powerful analytical tool for the clinical diagnosis of diabetes and played a key role in self-monitoring and management of blood glucose levels of diabetics. A biosensor is defined as "an analytical device consisting of a biological recognition element contacting with a suitable transducer, which is able to convert the biological recognition reaction or the biocatalytic process into a measurable signal". The transducer could be classified into electrochemical, optical, thermometric, piezoelectric, and magnetic types. Due to its better sensitivity, easy fabrication and low cost, electrochemical transducer-based blood glucose meter has dominated the glucose sensor market. The glucose biosensor technology developed quickly since its invention, and approximately 85% of the global biosensor market today is occupied by glucose-measuring devices, which accounts for $11.5 billion USD according to a recent report by Global Industry Analysts, Inc. The market is still growing rapidly and fierce competition is engaged by manufacturers. This article focuses on the current research trend and progress toward nanotechnology-based next generation blood glucose monitor. The history of glucose biosensor and our related work is briefly introduced and future prospects of the glucose sensor technology are also discussed in this editorial.

The first-generation glucose sensors were pioneered by Clark and Lyons in 1950s and 1960s.[1] Their device relied on a thin layer of glucose oxidase (GOx) enzyme, immobilized over an oxygen electrode (via a semipermeable dialysis membrane), and monitored the amount of oxygen consumed by the enzyme-catalyzed reaction by electrochemical method.[2] The technique was further developed by Updike and Hicks[3] who employed an additional oxygen working electrode (without enzyme) and measured the differential current between two working electrodes to correct for the oxygen background variation in samples. Subsequently, Guilbault and Lubrano[4] introduced another enzyme electrode for blood glucose measurement based on amperometric monitoring of the hydrogen peroxide as a product. The drawbacks of first-generation glucose sensor are strong dependence of ambient oxygen and too high applied (over) potentials, which may cause serious interfering reactions of electroactive compounds (e.g., ascorbate, urate and paracetamol) in the blood if a size-selective membrane is not present.

The second-generation glucose sensors were based on mediators and have been introduced in 1980s.[5, 6] Mediators were small, soluble redox active molecules (e.g., ferrocene derivates, ferrocyanide, conducting organic salts and quinones) capable of undergoing rapid and reversible redox reactions, shuttling the electrons between the redox center at the active site of enzyme and the electrode surface. Incorporation of mediators in glucose sensor alleviates the influence of interferential molecules and eliminates the dependence of oxygen for glucose sensing. Taking advantages of high specificity and reliability of second-generation glucose sensor, the first personal glucose meter has been commercialized by Medisense Inc. in 1987.[7] Various other companies including Roche Diagnostics, LifeScan, Abbott and Bayer thereafter have launched different types of glucose sensors with lower sample loading volumes and more advanced functions (Table 1), but the basic concept of glucose sensor design has remained largely unchanged. With large surface area and fast electron-transfer capability, nanomaterials have been increasingly studied to immobilize the

	LifeScan One Touch Ultra	Roche diagnostics Accu-check advantage	Bayer diagnostics glucometer Elite XL	TheraSense FreeStyle	MediSense precision QID
Alternate site testing	Yes	No	No	Yes	No
Sample size (µl)	1	3–4	2	0.3	3.5
Test time (s)	5	40	30	15	20
Capillary action strip	Yes	Yes	Yes	Yes	No
Temperature range (°C)	5–44	8–39	10–39	10–35	18–30
Test memory	150	100	120	250	10–125
Data downloading	Yes	Yes	Yes	Yes	Yes

Table 1. Characteristics of leading commercially available biosensors

enzyme in order to improve the sensitivity and response time of the glucose sensor. We fabricated a highly sensitive and stable glucose biosensor by immobilizing GOx on gold electrode modified with multilayered composite material of gold nanoparticles decorated carbon nanotubes.[8] The nanomaterial-based biosensor enables a high sensitivity and fast response time for the glucose detection. Although the practicability of second-generation glucose biosensor has been successfully achieved by the commercialization of a series of home-use, disposable devices based on screen printed strips, the soluble nature of most mediators leads to short operation time and irreproducible results, and the potential biotoxicity of mediators prevent the second-generation biosensor from application for *in vivo* glucose testing.

The ultimate goal of glucose sensing is to eliminate the usage of a mediator, even enzyme to lower the fabrication cost and complexity while increasing the durability of the glucose sensor. The third-generation glucose sensor enables direct electron transfer between the redox center of enzyme and electrode, leading to a very high sensitivity and reproducibility without using mediators. Due to a significantly lowered operating potential, the interferential responses of electroactive species are also greatly diminished. Nanoporous materials, especially the ones with the pore size similar to the dimension of GOx, could greatly facilitate the direct electron tunneling between the active site of protein and electrode.[9] Recently, we fabricated a mediatorless third-generation glucose sensor with nanoporous TiO_2 and GOx modified glassy carbon electrode.[10] The biosensor achieved a fast response time of 5s with a high sensitivity of 9.9 µA mM cm^{-2}. Different types of third-generation glucose biosensors have also been reported by my colleague Guo,[11, 12] who applied nanocomposite materials such as ZnO/carbon and NiO/conducting polymer to immobilize the enzyme and mediate direct electrochemistry. The third-generation glucose sensor is well suited for the *in vivo* monitoring of blood sugar due to its stability and biocompatibility. However, it suffers from relatively smaller linear range compared to the first- and second-generation glucose sensors. Therefore, the implantable glucose monitors on the market currently are still based on the concept of first-generation ones. Further effort is needed to improve the performance of the third-generation of glucose sensor in order to meet the commercialization criterion.

Another trend for glucose monitoring technology is the development of non-enzymatic glucose sensor with nanomaterials. This technology promises to overcome the problems associated with the enzymatic glucose sensor, i.e., the insufficient stability resulting from the intrinsic nature of enzyme. According to a review by Wilson and Turner,[13] GOx quickly loses its activity when it is exposed to the environment with pH below 2 or above 8, and temperature higher 40 °C. Ionic detergents also deactivate GOx significantly. Thermal and chemical instability of GOx prohibits enzymatic glucose sensors from being used for continuous monitoring glucose in human bodies. In addition to temperature, and pH, the response of the enzymatic glucose sensor is also affected by humidity. Either high or low humidity may harm the biosensors in use as well as storage. All these reasons are why nonenzymatic glucose sensors have received keen interest. Several nanomaterials, including nanopoprous platinum,[14] palladium/carbon nanotubes[15] and gold nanoparticle/graphene[16] have been reported to exhibit high sensitivity and selectivity toward enzymeless glucose detection. Recently, our colleagues Gao and Chen have reported two novel nonenzymatic glucose biosensors based on PtNi alloy nanoparticle/graphene composite[17] and cobalt oxide nanowire/3D graphene hybrid.[18] Their biosensors showed an improved sensitivity and stability toward glucose sensing. Despite of the advantages of enzymeless glucose sensor, there are some key issues in practical application. The first problem is the surface poisoning of electrode by absorbed intermediates, mostly chloride ions, which could quench the amprometric

signals during continued operation. The second issue is the toxicity of the heavy metal elements, some of which are not suitable for *in vivo* usage. Finally, since the enzymeless glucose sensor does not contain any glucose recognition element, the influence of interferents could be serious unless the glucose is oxidized on the electrode surface at significantly negative potential. This problem has been partially solved by previous studies. [15, 17]

In conclusion, this editorial briefly introduces the current research trend of glucose biosensors, and the related works in our lab as well as those in the School of Chemical and Biomedical Engineering at Nanyang Technological University. Although the glucose sensor research began as early as more than 60 years ago, there is no sign of illnesses until today. The advances of nanotechnology have significantly boosted research in biotechnology and biosensors. The breakthrough in the key performances of glucose biosensors, including higher sensitivity, better selectivity, faster response time, and smaller size are achieved with the help of nanomaterials. In addition, nanomaterials enable the fabrication of mediator-free and enzymeless glucose sensors, which have lower cost and higher stability than the traditional glucose meters and possess the advantages for *in vivo* glucose monitoring. Therefore, nanomaterial-based novel glucose biosensors are expected to have great market potential in the future.

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