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Nanotechnology in glucose monitoring: Advances and challenges in the last 10 years

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ABSTRACT

In the last decades, a wide multitude of research activity has been focused on the development of biosensors for glucose monitoring, devoted to overcome the challenges associated with smart analytical performances with commercial implications. Crucial issues still nowadays elude biosensors to enter the market, such as sensitivity, stability, miniaturisation, continuous and in situ monitoring in a complex matrix. A noteworthy tendency of biosensor technology is likely to push towards nanotechnology, which allows to reduce dimensions at the nanoscale, consenting the construction of arrays for high throughput analysis with the integration of microfluidics, and enhancing the performance of the biological components by using new nanomaterials. This review aims to highlight current trends in biosensors for glucose monitoring based on nanotechnology, reporting widespread representative examples of the recent approaches for nanobiosensors over the past 10 years. Progress in nanotechnology for the development of biosensing systems for blood glucose level monitoring will be discussed, in view of their design and construction on the bases of the new materials offered by nanotechnology.

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1. Introduction

Nanotechnology deals with the properties of materials at the nanoscale, at dimensions between approximately 1 and 100 nm,

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where particular physiochemical processes become more performant, such as improved plasticity, noticeable thermal and optical properties changes, higher reactivity and activity, faster electron/ion transport, novel quantum mechanical features (Vaddiraju et al., 2010). Nanotechnology provides a useful tool to acquire knowledge about biophysical phenomena at the nanoscale, and to project new materials with novel properties and functions for a wide range of applications. Among them, biosensors represent nowadays one of the main widespread result of this discipline (Jianrong et al., 2004), since interchangeable biocomponents, improved sensitivity and stability, miniaturisation and microfluidic integration can be provided (Fig. 1). In particular, biosensor research for glucose monitoring and diabetes management received enormous benefits from nanotechnology (Cash and Clark, 2010; Arya et al., 2008), concerning the design of new nanomaterials (such as electrodes, membranes, microfluidics, supporting hardware), the integration of nanostructured surface or nanomaterials able to improve biocomponents performances, and the construction of biocompatible and implantable devices for continuous monitoring.

In this review, progress in the development of glucose monitoring was described, specifically focusing on electrochemical and optical nanobiosensors, which were widely designed and realised over the last 10 years. Biosensors based on nanomaterials (nanoparticles, nanotubes, nanofibres, nanowires, and nanocomposites) were reported, highlighting the synergy between nanotechnology and enhanced biosensor performance. Finally, the challenges associated with the development of a nanobiosensor with a commercial success, such as sensitivity, stability, miniaturisation, continuous and in situ monitoring in a complex matrix, were reported.

2. Glucose monitoring

Glucose is a major source of energy for cells, and it is transported to cells via insulin in the bloodstream. The human body regulates blood glucose levels at a concentration of 4–8 mM (70–120 mg dL^{-1}). In the presence of physiopathological conditions blood glucose level could range in 2–30 mM (30–500 mg dL^{-1}). A persistent high glucose level is present in diabetic patients, since they are unable to regulate sugar level. Diabetes is a metabolic disease, resulting in an abnormal blood sugar level and consequently in the activation of several metabolic pathways related to inflammation and apoptosis events (Vashist et al., 2011).

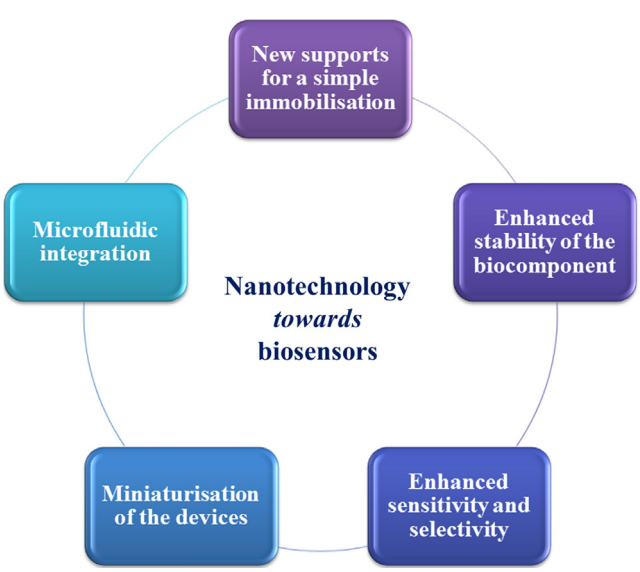

Fig. 1. Nanotechnology contributions for the development of biosensors with commercial promises.

Bibliography widely documented numerous important damages associated with long-term manifestations of diabetes, which represents a leading cause of mortality and several complications for human health, such as complications to retina, circulatory system, kidneys. According to a recent study (Shaw et al., 2010), the world incidence of diabetics (aged 20–79 years) are estimated at about 6.4% in 2010, and will increase to 7.7% by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of diabetic patients in developing countries and a 20% increase in developed countries. Moreover, the World Health Organization (WHO) has predicted that people with diabetes worldwide were approximately 171 million in 2000, and this is expected to increase to 366 million by 2030 (Narayan et al., 2006; Cowie et al., 2010; Shaw et al., 2010).

In order to manage blood glucose levels and therefore reduce hyper or hypoglycemia complications, the research community has spent important resources in the development of smart diagnostic tools for diabetes management (Yoo and Lee, 2010; D′Orazio, 2011). To achieve optimal control, patients need to monitor blood glucose level constantly, by painful sampling and obtaining great variations of the data. For these reasons, there has been an increasing demand for developing new techniques able to satisfy a continuous and non-invasive glucose monitoring with high accuracy, low cost, simplicity in sampling and testing, portability, reliability.

In this context, an immeasurable number of different biosensing approaches was provided for glucose monitoring, since the first biosensor designed by Clark and Lyons (1962) based on electrochemical transductions linked to enzymes, by placing glucose oxidase in solution between a membrane and an electrode.

2.1. Electrochemical biosensors

Most biosensors are based on electrochemical (amperometric, potentiometric, impedimetric or conductometric) transduction, employing glucose oxidase (GO) or glucose dehydrogenase (GDH), with different detection limits depending to the analysed matrices, from blood to interstitial fluids (Borgmann et al., 2011). These enzymes originally derived from *Aspergillus niger* and *Acinetobacter calcoaceticus*, and were employed as wild-type or engineered enzymes, with increased production yield, activity, stability and selectivity. The first generation electrochemical biosensor was based on the entrapment of glucose oxidase enzyme in polymers or membranes on a metal or carbon working electrode used as transducer, and linked to a mediator of electrons. The liberation of electrochemical species, as hydrogen peroxide, in the enzymatic reaction was measured at the working electrode surface. The main obstacles were related to the requirement of a high operation potential to obtain a high selectivity and to minimise the interference of endogenous species, such as ascorbic acid, uric acid, maltose, galactose, xylose, and lactose. In addition, the dissolved oxygen in biological fluids leaded to undesirable fluctuations of the analysis. For this reason, the second generation glucose biosensors provided several improvements in the substitution of oxygen with redox mediators, such as ferrocene, ferricyanide, quinines, tetrathialfulvalene (TTF), tetracyanoquinodimethane (TCNQ), thionine, methylene blue and methyl viologen, which were dispensed to carry electrons from the enzyme to the surface of the working electrode. The mediator was reduced in the reaction and reoxidised at the electrode, providing an amperometric signal and regenerating the oxidised form of the mediator. Finally, the third generation glucose biosensor allowed the direct electrical communication between the enzyme and the electrode surface. The absence of mediators permitted a higher selective and reagentless sensing, useful for implantable devices for continuous

in vivo monitoring of blood glucose. A representative scheme, which highlights the evolution of electrochemical biosensors technology in glucose monitoring over the years, was reported in Fig. 2.

2.2. Optical biosensors

Optical biosensors for glucose detection were also reported by several authors, by employing inactive apo-enzymes, binding protein, and receptors, which represented an alternative approach for the development of reversible, implantable and/or in line sensing systems (Scognamiglio et al., 2004, 2007; D′Auria et al., 2006; Herman et al., 2005; Jeffery, 2011). For example, in Scognamiglio et al. (2004), the use of inactive forms of apo-glucose oxidase from *A. niger* was described for the development of a reversible and non-consuming glucose sensor. The oxidase was rendered inactive by removal of the FAD cofactor, which is strongly bound to the protein structure as $FADH_2$-GO and reacts with O_2 to produce FAD-GO and H_2O_2. The resulting apo-GO was still able to bind glucose, with a K_d comparable to the holo-GO, without consuming it. Veetil et al. (2010) designed a new glucose sensor protein, AcGFP1-GBPcysmCherry capable of generating quantifiable Forster resonance energy transfer (FRET) signals for glucose monitoring, in response to glucose concentrations varying from 25 to 800 μM. In his design, Veetil fused a glucose-binding protein (GBP) with AcGFP1 at its N-terminus and mCherry at its C-terminus. The resulted structure was able to adopt an "open" conformation in the presence of glucose and a "close" conformation in the absence of glucose, allowing a change in the relative distance between the various components and consequently varying the FRET efficiency between them. A similar configuration was also described by Scognamiglio et al. (2007) in the development of a non-consuming analyte fluorescence biosensor to monitor glucose level in diabetes health care. In particular, a mutant form of a bacterial glucose-binding protein (GBP) was covalently labelled with a donor-acceptor pair fluorophores to set-up a FRET based sensing system for glucose. 6-Acryloyl-2-dimethylaminonaphthalene and rhodamine-isothiocyanate were selected for labelling the cysteine 182 (inserted by site-directed mutation) and the N-terminus, respectively. The distance between these fluorescent probes were founded to be 46.27 Å. Upon glucose binding, GBP undergoes a conformational modification capable to provide a rational placement of the fluorophores allowing a decrease of the distance up to 38.27 Å in the presence of glucose. This glucose-dependent spatial realignment of the two probes revealed to be adequate to develop a reasonable energy transfer, therefore attractive towards the set-up of a sensitive biosensor. In Fig. 3 a cartoon model of labelled GBP sensor is reported.

Detection of glucose was likewise accomplished by means of surface plasmon resonance (SPR) based biosensors (Tian et al., 2010; Baba et al., 2010), magnetic acoustic resonance sensors (MARS) (Hu et al., 2012), and calorimetric biosensors (Zhang and Tadigadapa, 2004). Any optical, acoustic or calorimetric sensing devices, in comparison with the electrochemical systems, has yet entered the market, but similar biosensors could help to develop non-consuming sensing systems, thanks to the use of receptor biocomponents.

3. Nanoscale biosensors

Although glucose biosensors show a long history, the realm of nanobiosensors is relatively new.

Nanoscale biosensors or nanobiosensors represent crucial steps in the aim of developing biosensing devices devoted to measure blood sugar level, to manage the health-care of diabetic patients and to follow-up diabetes disease, thanks to their numerous advantages (Jain, 2007). Nanobiosensors, provide to tune level of sensitivity as required by the analysis, and allow different detection limits for dermal interstitial fluids, urine, capillary or venous blood glucose monitoring. The use of nanomaterials functionalized with biocomponents can dramatically improve the stability and specificity of the detection system, yielding also reproducibility and reliability. Nanotechnology allows the miniaturisation and the integration of biocomponents, transduction systems, electronics and microfluidics in complex nanobiosensor architectures, able to perform both continuous glucose monitoring as implantable devices, and high throughput analyses as lab-on-chip devices for

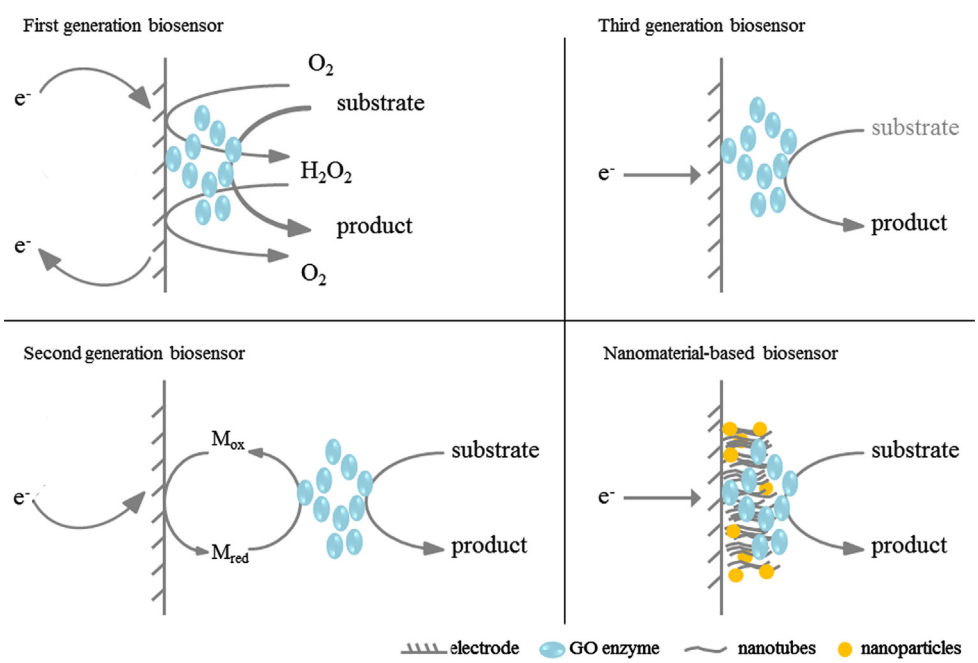

Fig. 2. Representative scheme of the three generations of amperometric glucose biosensors which highlights the evolution in biosensing research over the years, and of the last generation of biosensors employing nanomaterials for the direct biocomponent immobilisation.

rapid and low cost screening of glucose and a wide variety of physiological metabolites, using small samples of patient material.

Similar features have warmed a wide number of researchers to explore alternative strategies based on different nanomaterials, nanostructures or nanotechnologies, for the development of both electrochemical and optical biosensors. To date, modern nanomaterials have achieved high degree of complexity, in terms of synthesising functional tools with custom-made properties and controlled nanoscale. Numerous types of nanomaterials have been employed for biosensors development, from spheres and particles (metal nanoparticles, magnetic nanobeads, quantum dots), to nanotubes, nanowires, nanorods, nanofibres, as well as nanocomposites, nanofilms, nanopolymers, and nanoplates. These materials are able to enhance the performance of detection systems, thanks to their unique and special physical, chemical, mechanical, magnetic and optical properties, such as strong absorption band in the visible region, high electrical conductivity and good mechanical features (Zhang et al., 2009).

A huge literature documents the efforts and the challenges to realise nanomaterials for glucose sensing, with faster response time, higher storage/operational stability, resistance toward environmental conditions, improved selectivity, reduced blood sample volumes, and easy sampling.

3.1. Nanoparticles

Among nanomaterials, metal nanoparticles (NPs) have been largely employed in electrochemical biosensing systems as well as immunosensors, showing large signal enhancements and lower limits of detection (Table 1). Gold nanoparticles (AuNPs) may serve as surface for the attachment of biocomponents in platforms set-up, in self assembled monolayers (SAM) or for sandwich immunoassay. AuNPs have also been employed as fluorescence quenchers, thanks to their optical properties, in optical biosensors where the target analytes were labelled with gold nanoparticles, showing enhanced sensitivity towards targets by several order of magnitude (D′Orazio, 2011). Luo et al. (2004) demonstrated that the immobilisation of glucose oxidase enzyme on gold nanoparticles provides a higher stability of the biosensor in the time, since gold nanoparticles were able to strongly adsorb the enzyme and thus prevent the leakage of enzyme. In his study, a biocomposite film consisting of chitosan, glucose oxidase and AuNPs was electrochemically deposited on a gold electrode, during the electrodeposition, and the effect of gold nanoparticles on the enzyme immobilisation was studied. They compared the response of the biosensors prepared with and without gold nanoparticles to glucose, and stated that the biosensor with gold nanoparticles was more stable than that without gold nanoparticles. Zhang et al. (2005) also covalently attached glucose oxidase to a gold nanoparticle monolayer modified Au electrode, efficiently improving the electron transfer between analyte and electrode surface. By means of cyclic voltammetry and electrochemical impedance investigations, they demonstrated that the biocomponent was successfully and stably immobilised onto Au electrode, and the gold nanoparticles have a noticeable influence on the interface

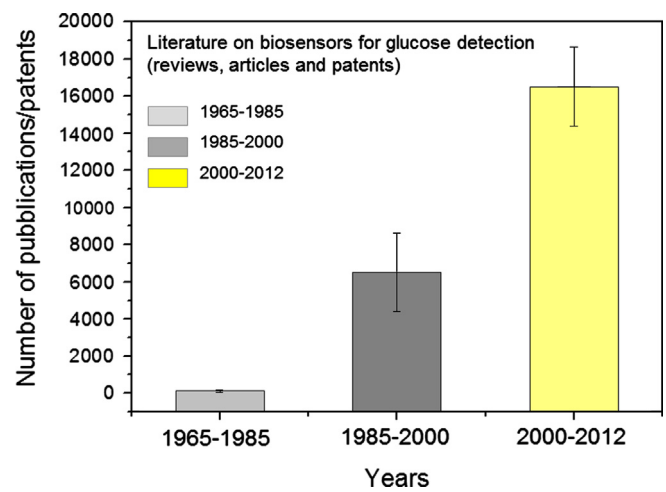

Fig. 4. Number of publications during the cited period relating to glucose biosensors. Data taken on October 2012 from www.googlescholar.com using the terms “glucose biosensors”.

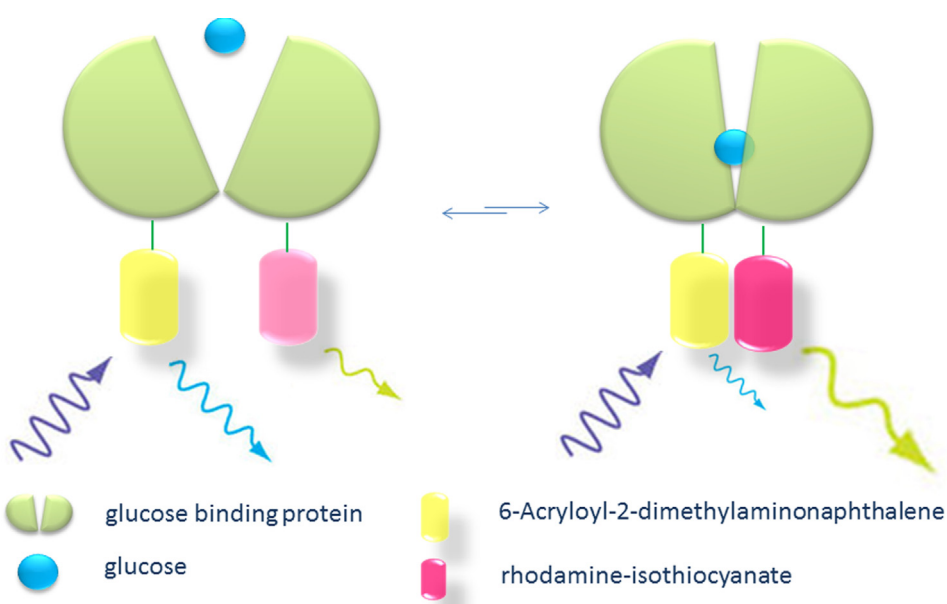

Fig. 3. Cartoon model of labelled GBP sensor. Fluorophores (Acryloyl-2-dimethylaminonaphthalene in yellow and rhodamine-isothiocyanate in pink) were attached on two different lobes of the binding protein, consisting of two lobes (green) connected by a hinge. Ligand binding transduces a hinge motion, propagated through a spatial realignment of the two probes, which reorient the relative excitation-emission dipole. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Some example of the main nanoparticles-based biosensors for glucose detection realised in the last 10 years.

Nanoparticles	LOD/detection range	Selectivity	Stability	Transduction system	Matrix	Ref.
Alkanethiol monolayer on silver film over nanosphere (AgFON) surface	0–250 mM	–	–	Surface-enhanced Raman spectroscopy (SERS)	–	Shafer-Peltier et al. (2003)
Chitosan hydrogel on gold nanoparticles	2.7 μM, 5.0 μM–2.4 mM	–	5 weeks	Electrochemical	Serum	Luo et al. (2004))
Gold nanoparticle monolayer modified Au electrode	8.2 μM, 2×10^{-5}–5×10^{-3} M	–	30 days	Amperometry	–	Zhang et al. (2005))
Iron oxide (Fe_3O_4) nanoparticles—chitosan (CH) solution—nanocomposite film on indium–tin oxide (ITO) glass plate	10–400 mg dL^{-1}	–	8weeks		–	Kaushik et al. (2008)
Exfoliated graphite nanoplatelets (xGnPs) decorated with Pt and Pd nanoparticles	1 μM	8% response with ascorbic acid 18% response with uric acid (56% and 125% without nanoparticles)	70% activity after 1 month 50% activity after 2 months	Amperometry	–	Lu et al. (2008)
Graphene and gold nanoparticles (AuNPs) on gold electrode	180 μM	good performance in human blood	2 weeks	Amperometry	Human blood	Shan et al. (2010)
Gold nanoparticles (Au-NPs)	0.1–0.08 mmol L^{-1}	–	19.5 days	Amperometry	–	German et al. (2010)
Chitosan/$NiFe_2O_4$ nanoparticles (CHIT/$NiFe_2O_4$NPs) on a glassy carbon electrode (GCE)	1×10^{-4}–2×10^{-2} mol L^{-1}	Any interferences from maltose, aldose, D-fructose, mannite, Fe3+, Ni2+, Cu2+, Co2+,Mn2+, Zn2+; serious interference from ascorbic acid and uric acid	30 days	Amperometry		Luo et al. (2010)
Palladium nanoparticles (PdNPs)-functioned graphene (nafion–graphene)	1 μM	Responce current: glucose 3.5 μA, ascorbic acid 0.08 μA, uric acid~0, acetamidophenol~0	35 days	Amperometry	Human serum	Lu et al. (2011)
Gold nanoparticles on eggshell membrane (ESM)	3.5 μM	Any interferences from sucrose, lactose, urea, glycine, citric acid, and NaCl; insignificant interference from ascorbic acid	10 weeks	Amperometry	Human blood serum	Zheng et al. (2011)
Platinum nanoparticles (PtNP) on mesoporous silica nanoparticles (MSN)	1×10^{-6}–2.6×10^{-2} mol L^{-1}	Free of interferences	90% activity after 1 month (used more than 100 times)	Amperometry	Clinical serum	Li et al. (2011)
Ordered mesoporous carbon (OMC) platinum nanoparticles (Pt/OMC) modified electrode	0.05 mM	Any interferences from ascorbic and uric acid	10 h operational stability; 2 weeks storage stability	Amperometry	Human serum	Jiang et al. (2011)
Poly(methyl methacrylate)-bovine serum albumin (PMMA-BSA) core-shell nanoparticles	0.2–9.1 mM	Any interferences from ascorbic and uric acid	1 month	Amperometry	Human serum	He et al. (2012)
Pt nanoparticles chitosan composite film (PtNPs-CS)	0.4 μM	Any significant interferences	1 month	Amperometry	–	Li et al. (2012)
PAH (poly(allylamine hydrochloride)) modified gold nanoparticles, deposited on planar interdigitated electrode (IDEs)	3 μM	Any interferences	12 days	Amperometry	Human blood serum	Nouira et al. (in press)
Imprinted hybrid microgel of Ag nanoparticles	0.1–20 mM	Low interferences from lactate	2 months	Surface plasmon resonance (SPR)	Tear fluids	Wu et al. (2012)
Hybrid nanogels of Ag nanoparticle cores covered by a copolymer gel shell of poly(4-vinylphenylboronic acid-co-2-(dimethylamino)ethyl acrylate) [p (VPBADMAEA)].	0–30 mM	Low interferences from lactate, any interferences from other sugars and ions	–	Optical	In vitro sensing and insulin release	Wu et al. (2010a, 2010b)
Gold nanoparticles (AuNPs)	0.008–4 mM 0.01–7 mM	–	–	Electrochemical Impedance Spectroscopy (EIS) and Surface Plasmon Resonance (SPR)	–	Bourigua et al. (2012)

property of the modified electrode and play an important role in improving the analytical performance of biosensor. Indeed, the analytical performance of the biosensor showed a linear response to glucose detection in a wide range between 2.0×10^{-5}–5.7×10^{-3} M, in addition to a stability of 30 days. German et al. (2010) studied the electrochemistry of glucose oxidase (GOx) as model to compare the performance of a graphite rod electrode biosensor in the absence and in the presence of gold nanoparticles. His study showed that the application of Au-NPs increases the rate of mediated electron transfer obtaining response to a glucose concentration in a linear range from 0.1 to 10 mmol L^{-1}, with a detection limit within 0.1 mmol L^{-1} and 0.08 mmol L^{-1}, and a storability up to 20 days.

Among the various type of nanoparticles, also the magnetic ones have gained great interest due to promising applications in biosensors. Iron oxide (Fe_3O_4) nanoparticles have been considered as interesting in particular for the immobilisation of biocomponents, thanks to their biocompatibility, strong superparamagnetic property, and low toxicity. Kaushik et al. (2008) prepared Fe_3O_4 nanoparticles to fabricate nanocomposite film of chitosan (CH) on an indium–tin oxide (ITO) glass plate, for the immobilisation of glucose oxidase via physical adsorption. He demonstrated that the novel composite of CH–Fe_3O_4 provided high accessibility to electrons between the enzyme and the electrode, improving electrocatalytic behaviour and consequently the biosensor performance in terms of repeatability and reliability. Chitosan/$NiFe_2O_4$ nanoparticles were used by Luo et al. (2010) for the immobilisation of glucose oxidase, showing excellent electrocatalytical response to the oxidation of glucose, when ferrocene carboxylic acid was used as artificial redox mediator by cyclic voltammetry. Platinum (PtNPs) and palladium (PdNPs) nanoparticles were used by Lu et al. (2008) in the fabrication of graphite nanoplatelets, in order to increase the electroactive area of the electrode and decreased the overpotential in the detection of hydrogen peroxide, obtaining a very fast biosensor (2 s in response), with low production costs. In addition, high selectivity was demonstrated for the nanoparticles-based biosensor, in comparison with the biosensor without nanoparticles. Indeed, addition of ascorbic acid or uric acid to glucose solution resulted in a 56% and 125% increase in the biosensor output, respectively, for the biosensor without nanoparticles. On the other hand, a 8% or 18% increase was provided for the nanoparticles-based biosensor, indicating an increased selectivity. Lu et al. (2011) similarly functionalized a nafion–graphene electrode with palladium nanoparticles, to obtain a non-enzymatic electrochemical biosensor for glucose, with very high electrochemical activity for electrocatalytic oxidation of glucose in alkaline medium, good reproducibility and long-term stability, as well as high selectivity with no interference from other potential competing species.

A different kind of nanoparticles was used by Li et al. (2011). In his study, amino group modified mesoporous silica nanoparticles (MSN) were involved to immobilise platinum nanoparticles and glucose oxidase, demonstrating high stability and reactivity for catalysing H_2O_2 electro-reduction, due to the large amount of PtNP immobilised and the high surface area of the unique nanostructures formed through the synthetic route.

On the other hand, Nouira et al. (in press) performed a deep study to demonstrate the feasibility and the performances of nanoparticle biosensing, by comparing two types of nanoparticles, gold and magnetic. He developed a glucose conductometric biosensor by immobilising glucose oxidase on poly(allylamine hydrochloride) (PAH) modified nanoparticles, deposited on a planar interdigitated electrode (IDEs), obtaining the best sensitivities with magnetic nanoparticles (70 μM/mM and 3 μM of detection limit) compared to 45 μM/mM and 9 μM with gold nanoparticles and 30 μM/mM and 50 μM with glucose oxidase directly cross-linked on IDEs.

The use of nanoparticles for optical sensing has also attracted growing research efforts, thanks to their unique optical features. Several research groups have reported optical SPR biosensors based on the extraordinary optical properties of metal nanoparticles to scatter light of different wavelengths. For example, Wu et al. (2012) projected an imprinted hybrid microgel made of Ag nanoparticles able to optically monitor glucose levels with high selectivity also in complex media at physiological pH. Indeed, the surface plasmon resonance response of the imprinted hybrid microgel enabled glucose detection without significant interferences, over a clinically relevant glucose concentration range of 0.1–20 mM.

Optical detection of glucose in a clinically relevant range (0–30 mM) and release of insulin were simultaneously performed by Wu et al. (2010a, 2010b), using a multifunctional hybrid nanogel made of Ag nanoparticle (NP) cores covered by a copolymer gel of poly(4-vinylphenylboronic acid-co-2-(dimethylamino) ethyl acrylate). The device was able to demonstrate the versatility of a multifunctional nano-object for simultaneous optical diagnosis, self-regulated therapy, and monitoring of the response to treatment. Haes and Van Duyne (2002) described the remarkable optical properties of triangular silver nanoparticles in the development of a glucose biosensor based on surface plasmon resonance spectroscopy, obtaining a substantial improvements in the detection limit, reduction in time response, and enhanced selectivity. A hybrid biosensor based on gold nanoparticles was instead reported by Bourigua et al. (2012), for the detection of glucose by means of a double transduction which combined electrochemical impedance spectroscopy and surface plasmon resonance, obtaining a high sensitive and a wider dynamic range of detection.

3.2. Nanowires, nanofilms and nanofibres

Nanowires, nanofilms and nanofibres also show exclusive physical and electronic features in terms of electron transfer and high surface exposure. Recent results (Table 2) have suggested that these nanostructures can be used as supports for biomaterial absorption, providing high loading and responsive microenvironment to stabilise the immobilised molecules. In addition, these materials can be incorporated in high-density arrays, providing large surface area, high current density, fast electron transfer, and enhanced sensitivities. Presently, increasing researches have been committed on nanomaterials, resulting in a remarkable improvement of glucose biosensors performance. Nanowires-based biosensors were developed by Wang et al. (2009), Usman Ali et al. (2010), Wang et al. (in press), and Pradhan et al. (2010), by immobilising glucose oxidase on nanowires, increasing enzyme absorption and electron transfer with the surface, and obtaining high sensitivity, low detection limit, fast response, and good stability of the sensor. Xu et al. (2012) designed electrochemical polymerizated polypyrrole (PPy) nanowires, with a 20 nm diameter, to construct an array of aligned nanowires for the entrapment of glucose oxidase. He demonstrated how the nanowire array configuration allows a higher effective surface area for enzyme immobilisation, with respect to the traditional conductive polymer films.

Nanofibres, together with other conducting polymer nanostructures, including nanorods, and nanotubes, have also been widely employed in biosensors as materials which show the advantages of low-dimensional systems and organic conductors. Nanofibres have special electronic, magnetic, and optical properties, thus they have been used for several applications, from electrodes to capacitors, or as conducting molecular wires for the immobilisation of biological material for sensors. Several research activities have been focused on application of nanofibres to immobilise glucose oxidase, to facilitate the direct electron transfer of the enzyme. Ahmad et al. (2010) fabricated, by electrospinning

Table 2
Some example of the main nanomaterials-based biosensors for glucose detection realised in the last 10 years.

Nanowires, nanofilms and nanofibres	LOD/detection range	Selectivity	Stability	Transduction system	Matrix	Ref.
Gold nanoparticles-modified Pb nanowires	2 μM	Slight interference in diluted blood samples	70 days	Chronoamperometric	–	Wang et al. (2009)
ZnO nanowires	0.5–1000 μM	Not substantial interference from ascorbic and uric acid	–	Potentiometric	–	Usman Ali et al. (2010)
Silver Nanowires	2.83 μM	Negligible interference from ascorbic and uric acid	10 days	Electrochemical impedance spectroscopy (EIS)	Human blood serum	Wang et al., (in press)
ZnO nanowires (NWs) deposed on Au- coated polyester (PET)	19.5 μA mM^{-1} cm^{-2}	–	7 days	Amperometric	–	Pradhan et al. (2010)
Polypyrrole (PPy) nanowire arrays	50 μM	–	–	Amperometric	–	Xu et al. (2012)
Nanofibers (NFs) of poly(vinyl pyrrolidone)/zinc acetate composite	1 μM	Good anti-interference ability	4 months	Electrochemical	–	Ahmad et al. (2010)
Palladium nanoparticles on poly(3,4-ethylenedioxythiophene) nanofibers	1.6 mA M^{-1} cm^{-2}, 0.5–30 mM	Insusceptible to electroactive interfering species	12 days	Amperometric	Diluted human serum sample	Santhosh et al. (2009)
Vertically aligned carbon nanofiber	40 μM	–	–	Amperometric	–	Islam et al. (2009)
Vertically aligned carbon nanofibers (VACNFs)	0.4 μM	Any interference from phenols, aromatic amines, ascorbic acid, ferrocyanide andiodine	–	Amperometric	–	Islam et al. (2011)
Mn_2O_3–Ag Nanofibers	1.73 μM	Negligible interference from ascorbic and uric acid	–	Amperometric	–	Huang et al. (2011)
Gold Nanoparticles-Bacteria Cellulose Nanofibers Nanocomposite	2.3 μM	Any interference from citric acid, L-lysine, oxalic acid and sucrose	1 week	Amperometric	Human blood samples	Wang et al. (2010)

technique, poly(vinyl pyrrolidone)/zinc acetate composite nanofibres with diameters in the range of 350–195 nm. Each nanofibre was then immobilised on a gold electrode for the functionalization with glucose oxidase by physical adsorption, for the development of a highly sensitive glucose electrochemical biosensor. His work demonstrated that this nanomaterial can provide favourable stability and long-term storage (more than 4 months), as well as a good anti-interference capability. Poly(3,4-ethylenedioxythiophene) (PEDOT) nanofibres were electrodeposited by Santhosh et al. (2009), and used for the deposition of palladium nanoparticles and glucose oxidase. Co_3O_4 nanofibres were electrospinning fabricated by Ding et al. (2010), to construct a non-enzymatic sensor for glucose detection, showing high sensitivity, reproducibility and selectivity to detect glucose in human blood serum samples. For the same purpose, carbon nanofibres were employed by Islam et al. (2009, 2011). Mn_2O_3–Ag nanofibres were used by Huang et al. (2011) as immobilisation matrix for glucose oxidase, showing their great potential application in oxygen-reduction based glucose biosensing. Vamvakaki et al. (2006) reported in their study the main advantages of carbon nanofibres, with respect to carbon nanotubes or graphite powder, in the fabrication of biosensors. In particular, he prepared different grades of nanofibres with very low electrical resistivities and high ordered structures, with minimum spacing between the graphene layers. Wang et al. (2010) deposed a gold nanoparticles–bacterial cellulose nanofibres (Au–BC) nanocomposite on a glassy carbon electrode, for the immobilisation of glucose oxidase and horseradish peroxidase enzymes, to amperometric determine glucose in human blood samples, in the presence of an electron mediator. Wu et al. (2009) presented a refractometric sensor based on nanofibres to measure the refractive indices of glucose solutions of different concentrations, with very high stability and reliability.

3.3. Nanotubes

Similarly to other nanomaterials, nanoscale dimensions of nanotubes, together with their surface chemistry and electronic properties, have pushed the research community to consider them as ideal materials for use in chemical and biochemical sensing. These nanostructures have attracted great attentions as nanoscale building blocks for the construction of micro and nano-devices, since they allow biocomponent loading on higher surface area and consents higher conductivity, improving electrical communication between surfaces and immobilised biocomponents.

The combination of nanotubes with redox active enzymes has provided the development of suitable platforms in reagentless biosensors and nanobiosensors (Table 3). In this context, several electrochemical biosensing systems were described, based on electrodes coated with both single-wall nanotubes (SWNTs) and multiwall carbon nanotubes (NWNTs), and on carbon nanotubes (CNTs) arrays. These configurations have showed enhanced catalytic signal by several order of magnitude compared to that observed for macro-carbon electrode. Glucose oxidase immobilisation on carbon nanotubes was reported by several authors (Holzinger et al., 2011; Janegitz et al., 2011; Karachevtsev et al., 2012; Jose et al., 2012), which described how the electron transfer between the enzyme and CNT resulted feasible at low overpotentials, signals from electrochemically active interferences were reduced, and the response time was minimised compared to an equivalent biosensor without nanotubes. The electron transfer ability of MWNTs has been exploited by Wang et al. (2003), Salimi et al. (2004), Tsai et al. (2005), Norouzi et al. (2010), Fu et al. (2011) and Dalmasso et al. (2012), in the fabrication of sensitive and stable biosensors for detecting glucose, indicating that MWNTs are good candidate materials for enzyme immobilisation. Single walled nanotubes (SWNTs) were employed by Zafar

Table 3
Some example of the main nanotubes-based biosensors for glucose detection realised in the last 10 years.

Nanotubes	LOD/detection range	Selectivity	Stability	Transduction system	Matrix	Ref.
Carbon nanotube (CNT) nanoelectrode ensembles (NEEs)	0.08 mM	No interference was observed at a potential of −0.20 V from ascorbic and uric acid	–	Amperometric	–	Lin et al. (2004)
Carbon nanotubes/chitosan	0.52 μA mM^{-1}	Any interference from l-ascorbate, p-acetaminophenol and cysteine	15 days	Amperometric	–	Liu et al. (2005)
Gold/multi-walled carbon nanotubes (Au/MWNTs)	0.05–13 mM 0.01 mM	–	3 months	Amperometric	–	Wang et al. (2003)
Sol–gel composite on plane pyrolytic graphite (bppg) electrode modified with multiwall carbon nanotube	50 μM					Salimi et al. (2004)
Electropolymerized polypyrrole film doped with carbon-nanotube (CNT)	0.2 mM	–	Storage 1 week operational 3 min	Chronoamperometric	Serum samples	Wang and Musameh (2005)
CNT–cationic polydiallyldimethylammonium chloride (PDDA)	7 μM	No interference was observed at a potential of −0.1 mV from uric acid, ascorbic acid, and acetaminophen	4 weeks	Amperometric	–	Liu and Lin (2006)
Multiwalled carbon nanotubes (MWNTs)	4 μM	–	–	Amperometric	–	Tsai et al. (2005)
Gold and platinum nanoparticles–carbon nanotube (CNT) electrode	0.5–17.5 mM	Any interference from ascorbic acid and uric acid with Nafion film-coated electrode	25 days	Chronoamperometric	Human plasma samples	Chu et al. (2007)
Chitosan (CS) sol–gel deposed on multi-walled carbon nanotubes hybrids (PB/MWNTs)	7.5×10^{-6} M	No apparent influence in the presence of ascorbic acid and uric acid	36 h	Chronoamperometric	–	Fu et al. (2011)
Screen-printed electrodes made of carbon (SPCEs), carboxyl-functionalized single-walled carbon nanotubes (SPCE-SWCNTs), multiwalled carbon nanotubes (SPCE-MWCNTs)	10 μM	Working potential (0.1 V) partially minimise the contribution of interferences	Operational 7 h	Amperometric	–	Zafar et al. (2012)
Multiwall carbon nanotubes (MWCNTs) dispersed in polyhistidine (Polyhis) on glassy carbon electrodes (GCE)	2.2 μM	No apparent influence from other sugars	15 days	Amperometric	–	Dalmasso et al. (2012)

et al. (2012), where a direct electron transfer with the redox active centres of adsorbed oxidoreductase enzymes was described conceivable. In particular, he described a third-generation amperometric glucose biosensor based on a thermophilic dehydrogenase immobilised on carboxyl-functionalized single-walled carbon nanotubes. He also reported that the biosensor based on SWCNTs showed higher sensitivity and catalytic response than the one functionalized with MWNTs and carbon screen printed electrodes (SPEs), with very low electrochemical interferences by other carbohydrates such as mannose, galactose, sucrose, and fucose that might be present in blood.

The advantages of nanotubes in the construction of optical nanosensors were reviewed by Barone et al. (2009), in comparison to the traditional organic and nanoparticle fluorophores, especially for in vivo-sensing applications. Yoon et al. (2011) explored the direct coupling of a bacterial glucose-binding protein (GBP) to near-IR fluorescent SWNTs, to create a new type of optical sensor for glucose, combining the high selectivity of GBP with the special optical properties of nanotubes.

Yum et al. (2012) asserted that the fluorescence of SWNTs is highly responsive to their physical and chemical environment, making SWNTs a highly sensitive platform for biological and chemical sensing. He indeed described a reversibly glucose binding via a change in SWNT fluorescence, by using a library of 30 boronic acid derivatives to form complexes with sodium cholate suspended single-walled carbon nanotubes.

3.4. Nanocomposite

Aside from single nanostructure, nanocomposite structures, involving different kind of nanomaterials from particles to tubes, wires or fibres, are also widely utilised in biosensor configuration (Table 4). For example, Yang et al. (2004) constructed a nanoporous ZrO_2/chitosan composite matrix to fabricate a glucose biosensor, combining the advantages of inorganic nanoparticles, ZrO_2, and organic polymers, chitosan. The nanocomposites implemented by Yang conferred to the enzyme an activity 5 times greater than the glutaraldehyde cross-linked enzyme.

A vast plethora of biosensors was described based as well on composite nanostructures, frequently constituted by the arrangement of nanoparticles and nanotubes, such as the biosensing systems reported by Lim et al. (2005), Chu et al. (2007, 2010), Kang et al. (2007), Norouzi et al. (2010), and Sharma et al. (2012). These systems were constructed by employing the surface of carbon nanotubes as support for the highly dispersed immobilisation of gold or platinum nanoparticles. In similar configuration advantages of CNTs and nanoparticles are combined. In particular, the unique physical and electronic properties of nanotubes, such as the high electronic conductivity, the high surface/volume ratio and the exclusive skill to promote electron transfer, combined to excellent catalytic activity towards H_2O_2 of precious metal nanoparticles, that can reduce the oxidation/reduction overvoltage of hydrogen peroxide, have demonstrated to improve the electrochemical behaviour of the biocomponents.

Maniruzzaman et al. (2012) described a different kind of nanocomposite, in the fabrication of a titanium dioxide (TiO_2)–cellulose hybrid nanostructure, showing its high potential in the realisation of a flexible conductometric biosensor for the detection of glucose. Even, the biosensing application of single-walled carbon nanohorns (SWCNHs) was demonstrated by Liu et al. (2008) through the fabrication of a Nafion–SWCNHs composite film for the encapsulation of glucose oxidase. The resulted nanostructure, thanks to its high purity and high surface area, showed good electrocatalytic activity toward oxidation of glucose, as well as high sensitivity and stability. These concept were also described by Shan et al. (2010), in the realisation of a graphene/AuNPs/

Table 4
Some example of the main nanocomposite-based biosensors for glucose detection realised in the last 10 years.

Nanocomposite	LOD/ detection range	Selectivity	Stability	Transduction system	Matrix	Ref.
Palladium nanoparticles onto a Nafion-solubilized carbon nanotube (CNT) film	0.15 mM	Extra Nafion coating eliminates common interferents, such as uric and ascorbic acids	14 days	Voltammetric	–	Lim et al. (2005)
Nanogold colloids assembled on a polytyramine-modified gold electrode	1×10^{-6} M	Small sugars partially interfere, including fructose, mannose, maltose, glucopyranoside, mannopyranoside	1 day	Capacitive	–	Labib et al. (2010)
Gold nanoparticles (AuNPs) and multiwall carbon nanotubes (MWCNTs)	0.03 μM	Nafion films to prevent interference	30 days	Voltammetric	–	Norouzi et al. (2010)
Platinum (Pt) nanoparticles/polymerised ionic liquid-carbon nanotubes (CNTs) nanocomposites (PtNPs/PIL-CNTs)	10 μM	No obvious interference from uric acid and ascorbic acid	1 month	Amperometric	Blood samples	Chu et al. (2010)
Polypyrrole nanowires array (PPyNWA) with Pt nanoparticles (PtNPs)	5.6 μM	–	–	Amperometric	-	Xu et al. (2012)
Colloidal gold modified carbon paste electrode	0.01 mM	Any interference from uric acid and ascorbic acid	10 days	Amperometric	Serum sample	Liu and Ju (2003)
Platinum nanoparticle-modiWed carbon nanotube (CNT) electrode	0.1–13.5 mM	Thin layer of Nafon to improve the anti-interferent ability	22 days	Amperometric	Human plasma samples	Tang et al. (2004)
Biopolymer chitosan (CS) on glassy carbon electrode (GCE) modified with gold-platinum nanoparticles (Au-PtNPs) deposed on multiwall carbon nanotubes (CNTs) in CS film (CNTs/CS)	0.2 μM	Negligible response signals of uric acid and ascorbic acid	35 days	Amperometric	Human blood and urine samples	Kang et al. (2007)
Alkanethiolate tri(ethylene glycol) monolayer	0–25 mM	Not affected by the presence of large molecules, such as serum albumin	Operational 3 days	Surface-Enhanced Raman Scattering	Blood serum protein mimic solution	Yonzon et al. (2004)
Sol–gel/chitosan	0–20 mM	Any significant interference from L-cysteine hydrochloride, acetaminophen and uric acid	30 days	Amperometric	–	Chen et al. (2003)
Nanoporous ZrO_2/Chitosan composite matrix	1×10^{-5} M	Interference response from uric and ascorbic acid in the absence of Nafion	30 days	Amperometric	Blood samples	Yang et al. (2004)
Carbon nanofibres	0–8 mM	–	Operational 100 h	Voltammetric	–	Vamvakaki et al. (2006)
Mesocellular carbon foam	0–2 mM	–	10 days	Amperometric	–	Lee et al. (2005)
Polyvinylpyrrolidone-protected graphene	0–14 mM	Slight interference at −0.49 V potential	1 week	Voltammetric	–	Shan et al. (2009)
Bionanocomposite film Pt/functional graphene sheets/chitosan (Pt/FGS/chitosan)	0.6 μM	Negligible interference from uric and ascorbic acid	2 weeks	Amperometric	–	Wu et al. (2009)
Single-walled carbon nanohorns (SWCNHs)—Nafion	0–6 mM	Lactate, glutathione, cysteine, ascorbate and aminophenol not interfere	2 weeks	Amperometric	Blood samples	Liu et al. (2008)
Graphene oxide nanosheets	28 mM mm^{-2}	–	7 days	Amperometric	–	Liu et al. (2010)
Titanium dioxide (TiO_2)-cellulose hybrid nanocomposite	1–10 mM	–	36 h	Amperometric	–	Maniruzzaman et al. (2012)
Pt nanoparticles chitosan composite film (PtNPs-CS)	0.4 μM	Not significant interference from ascorbic acid, threonine, L-cysteine and uric acid	3 weeks	Amperometric	–	Li et al. (2012)
Graphene/AuNPs/chitosan nanocomposites film	2.5–7.5 mM	–	2 weeks	Voltammetric	Human blood	Shan et al. (2010)
CdS quantum dots (QDs) incorporated in poly(N-isopropylacrylamide-acrylamide-2-acrylamidomethyl-5-fluorophenylboronic acid) copolymer microgel	0–10.8 mM, 0.5 mM	Slight interference from lactate and Human serum albumin	–	Fluorescence	-	Wu et al. (2010a, 2010b)
Silica film (ORMOSIL) doped with tris(4,7-diphenyl-1,10-phenanthroline) ruthenium dichloride (Ru(dpp)3Cl2)	0.1–5.0 mM (0.06 mM)	No distinct interference from ions, ascorbic and uric acid	60 days	Fluorescence	Factual sample	Changa et al. (2010)

chitosan-modified electrode in comparison with a graphene/chitosan-modified electrode. The nanocomposite electrode employing gold nanoparticles was able to show an excellent reduction toward O_2 at −0.2 V with more positively reductive peak potential compared to the electrochemical reduction to O_2 at −0.3 V for the electrode without nanoparticles.

Wu et al. (2010a, 2010b) described a new class of optical glucose nanobiosensors based on the fluorescent CdS quantum dots (QDs), incorporated into a glucose-sensitive poly(N-isopropylacrylamide-acrylamide-2-acrylamidomethyl-5-fluorophenylboronic acid) copolymer microgels. He reported that the polymeric gel was able to adapt to surrounding glucose concentrations, and regulate the fluorescence of the embedded QDs, converting biochemical signals into optical signals. Moreover, the analytical biosensor performance, in terms of stability, reversibility, and sensitivity, was well tunable under appropriate tailor on the implemented hybrid nanocomposite, simply through the change in the crosslinking degree of the microgels. An optical glucose biosensor was also fabricated by Changa et al. (2010) by encapsulating glucose oxidase in an organically modified silica film (ORMOSIL) doped with tris(4,7-diphenyl-1,10-phenanthroline) ruthenium dichloride (Ru(dpp)3Cl2) as an luminescent oxygen transducer, for determining the concentration of glucose in blood and urine samples. Glucose oxidation was determined through increase in the fluorescence intensity of $Ru(bpy)_3Cl_2$ due to the oxygen consumption. The nanocomposite based biosensor showed good response performance to glucose with high sensitivity, good reproducibility and fast response.

4. Nanobiosensor performance issues

4.1. Miniaturisation

The prospects of nanobiosensors to be employed in hand-held devices, as well as for continuous monitoring or implantable devices, can be reached through the miniaturisation of the functional components, such as electrodes, power sources, signal processing units and sensory elements, as well as their subsequent integration and packaging. In this context, nanotechnology is endowed to achieve components miniaturisation and integration. The nanoscale dimensions of several nanomaterials, like nanoparticles, nanotubes, nanorods, nanowires and nanopolymers described before, together with their special physico-chemical properties and high surface area, have prompted to the realisation of smart supports for biological recognition elements immobilisation, with real advantages in enhancing biocomponents performance. For example, the nanoscale dimensions of nanotubes combined with the electrocatalytic properties and high surface area have driven several researchers to employ them as nanoelectrodes, as reviewed by Kim et al. (2007). In a nanobiosensor configuration, each nanotube works as an individual nanoelectrode and the small space between nanotubes is sufficiently able to prevent diffusion within neighbouring electrodes, providing good signal-to-noise ratio and improved detection limits.

With respect to miniaturisation of electronics, the nanofabrication through lithography techniques provided a powerful tool for submicron construction. Micro and nanofabrication by means of photolithography, dip-pen nanolithography and micromachining has allowed sensor miniaturisation, as described by Zine et al. (2003). He constructed a solid-contact ion-selective microelectrode (SCISME) based on silicon needles, for metabolites monitoring during cardiac surgery or during organ transportation for transplants, showing the feasibility of the fabrication technology and the high performance of the sensor device in terms of sensitivity and selectivity, also against common interfering substances. In Fig. 5 a schematic view of the needle-shaped silicon substrate for microelectrodes designed by Zine et al. (2003) is reported.

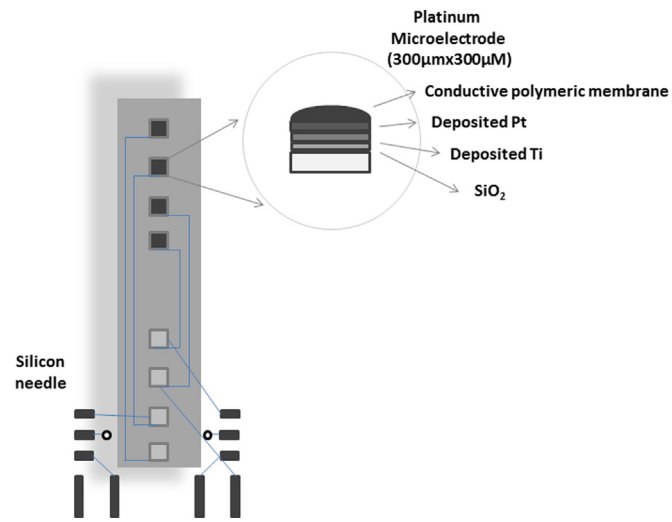

Fig. 5. Schematic view of the needle-shaped silicon substrate for microelectrodes designed by Zine et al. (2003). In the inset a cross-section of a microelectrode. Four platinum microelectrodes were fabricated in a silicon needle. The dimensions of each electrode were 300 μm×300 μm. On the platinum substrate of each microelectrode a layer of conducting polymer of 10 μm thickness and a layer of PVC of 100 μm thickness were deposited.

Needles and channels for fluid flow are also shaped by etching processes, as well as working and reference electrodes patterned by photolithography. Similarly, various functional electronic components, such as capacitors, photodiodes, filters, are micro/nanofabricated. Moreover, nanotechnology can provide advances in battery technologies and power generating circuits, by means of novel nanomaterials and fabrication methodologies for inductive coupling, variations in electromagnetic fields, electrostatic conversion of mechanical vibrations, and capacitance changes due to external vibrations. For example, inorganic nanowires and carbon nanotubes were employed in transistor configurations, or as interconnects, by several authors (Appenzeller et al., 2004). In addition, nanocomposites based on various nanomaterials may have the advantage to alter the surface charge, hydrophilicity and tensile strength of the sensing devices in order to prevent biofouling events. Roberts et al. (2012) tested three types of hydrogel materials, poly(2-hydroxyethyl methacrylate) (pHEMA), poly(acrylamide) (pAM), and poly(2-hydroxyethyl methacrylate)-co-poly(acrylamide) (p(HEMA-co-AM)), to analyse glucose diffusivity in a glucose monitoring implanted in rats for 1 month. The author asserted that biofouling, which is expected to affect the response of flux-based sensor, depends on the type of materials employed and suitable materials selection may minimise response alterations occurring upon implantation.

Finally, the soft and flexible nature of several nanomaterials, such as for example nanopolymers, could allow the minimisation of foreign body response or tissue damage, which may have important implications in implantable device design.

4.2. Sensitivity and selectivity

Sensitivity and selectivity are imperative matters for a biosensor. These parameters are correlated, since methodologies which increase sensitivity usually reduce selectivity. In this sense, nanotechnology can improve selectivity and sensitivity by means of new physical design of the sensor, by employing novel material able to enhance biocomponents activity or to amplify the surface of the working electrode (i.e. nanoparticles, nanotubes), and by

using polymer membranes to avoid interferences or prevent biofouling. The use of such nanomaterials forces to apply high potentials (i.e. 0.6–0.7 V vs. Ag/AgCl reference electrode) for electrochemical oxidation of enzymatically generated H_2O_2 in many cases of electrochemical biosensors. These potential values also promote the oxidation of many endogenous species, such as ascorbic acid, uric acid, hormones, altering the sensor response. In this context, nanotechnology proceed to offer smart nanomaterials, such as single wall nanotubes (SWNTs) (Zafar et al., 2012), multi walls nanotubes (MWNTs) (Norouzi et al., 2010) or carbon nanotubes (CNTs), capable to lower the potential, avoiding interferences and consequently improving selectivity. In addition, nanoscale biosensors are able to fine tune limits of detection, thanks to the intimate integration of the biocomponent with the nanosurfaces, or through the set-up of arrays of biocomponents to obtain cumulative and large responses. Indeed, in the aim of glucose monitoring, the goal is to reach the desired detection limit in dependence on the analysed matrices (interstitial fluids, urine, capillary, tear, or venous blood), on their volume, or on their diluted/pre-treated solutions. As an example, the typical range of glucose concentrations in the tear film is 50–500 μM, which track blood glucose levels are typically ≈5–10 fold higher (Badugu et al., 2003). Yao et al. (2011) reported the design, construction, and testing of a contact lens with an integrated amperometric sensor able to detect glucose in a minimum detection of less than 0.01 mM.

4.3. Continuous monitoring

Many research efforts have been focused on the design of sensing systems for continuous monitoring of metabolites of high biomedical interest, such as glucose. Nowadays, the current method to measure blood glucose occurs through a finger stick and subsequent glucose concentration measurements. This procedure is painful and unable of reflecting the trends of daily human habits, contributing, in this way, with only few measurements per day. In addition, this analysis depends from a number of parameters which can affect with a correct glucose concentration measurement, such as electrochemical interferents, temperature dependence, skin contamination with glucose or other sugars, presence of time-lag between venous glucose level and finger sites. Implantable biosensors could offer the opportunity to monitor glucose continuously without the need for patient involvement, and could be combined with microfluidic systems for glucose/insulin management. On the other hand, implantable devices are often affected by physiological factors like interference with other chemical species, extraneous body response, biofouling. To mitigate these issues, nanotechnology has led to the development of diagnostic devices by means of new inert materials and smart nanostructures for small and reliable glucose monitoring systems (Vaddiraju et al., 2010). For example, Chaudhary et al. (2010) designed and tested an implantable microparticle optical sensor for in vivo glucose monitoring by a fluorescence resonance energy transfer (FRET)-based competitive binding assay. He deposited a multilayered nanofilms on the alginate microspheres containing the assay and analysed response to glucose in water and simulated interstitial fluid (SIF) using a flow cell. Finally, he performed an in vitro cytotoxicity assay on L929 mouse fibroblast cell lines demonstrating that the employed materials showed sufficient biocompatibility for use as implantable biosensors. Paek et al. (2013) developed an implantable sensor by placing an optical fibre probe within the internal hollow space of a syringe needle. Concanavalin A was immobilised on the probe tip and bovine serum albumin coupled with mannose was loaded inside the needle, then locked by a semi-permeable membrane. Upon immersion in samples, glucose molecules were able to freely pass

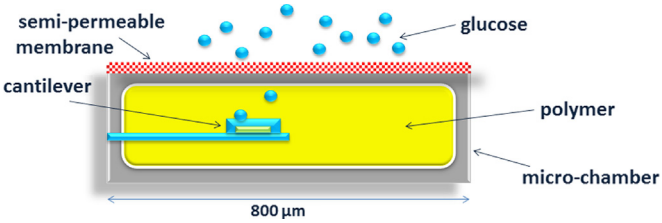

Fig. 6. Schematic view of the MEMS affinity CGM sensor design by Huang et al. (2009). A cantilever vibrates in a polymer solution sensitive to glucose inside a microchamber. Glucose permeates through a semi-permeable membrane, changing the solution viscosity and vibration damping.

through the membrane and compete with the ligand conjugate for Con A binding. This sensor configuration was able to monitor glucose in serum medium, with a response time of less than 15 min in a range 10–500 mg/dL. Finally, the authors advised a shorter response time of 5 min upon miniaturising of the sensor dimensions. Huang et al. (2009) presented a MEMS affinity sensor for long-term continuous monitoring of glucose in subcutaneous tissue. The described sensing principle was based on detection of viscosity changes due to affinity binding of a biocompatible polymer (poly(acrylamide-ran-3-acrylamidophenylboronic acid) (PAA-ran-PAAPBA)) towards glucose. The viscosity variations were sensed by means of a magnetically driven vibrating microcantilever situated in a microchamber filled by PAA-ran-PAAPBA. A semi-permeable membrane separate the polymer from the surroundings. Upon permeating, glucose reversibly bind the phenylboronic acid moiety of the polymer resulting in a viscosity change and consequently in a cantilever vibration. In Fig. 6 a schematic view of the MEMS affinity CGM sensor design by Huang et al. (2009) is reported. Moreover, nanotechnology could provide materials with special features to produce electronics with low energy consumption and battery with high energy capacity, essential for implanted devices.

4.4. Biocompatibility of nanomaterials

Taking into account the premise of nanotechnology to expand the commitment of biosensors for implantable uses, it became crucial to consider the toxicity and the biocompatibility of nanomaterials for in vivo monitoring. There are a number of points to discuss concerning nanomaterials. Among them, nanoparticles could enter the human body and interfere with biological processes, causing potential health risks, or nanostructures could determine inflammation and foreign body response (Ainslie and Desai, 2008). For this reason, several studies have been accomplished to estimate the immunogenicity of nanoparticles, to deduce the transport/uptake of nanomaterials in proximity of the biosensor, as well as to develop different approaches to overcome these issues. As an example, covalent attachments of nanomaterials to the functional component of the device can reduce the risk of leaching, thus excluding potential harmfulness.

5. The nanobiosensor trade

Although the high amount of research literature on biosensors for glucose monitoring, only sporadic devices succeed in reaching the commercial acceptance. Actually, the blood glucose monitoring trade represents the major driving force (over 85%) in the direction of commercial handheld biosensors. Many companies, well-known worldwide, have commercialised biosensing devices for glucose detection (Yoo and Lee, 2010). Therefore, there is a potential market still to be established. In this perspective, nanotechnology could pave the way to enhance biosensors performance, in order to gain

real applications in biomedical field, as well as in areas such as food, agriculture, military, veterinary, and environment. Indeed, thanks to important progresses in micro and nanofabrication, several laboratory analytical instruments, such as SPR technology, miniaturised mass spectrometry, chromatography or electrophoresis chips, were adapted to commercial requirements, becoming smaller and equipped with fluidics and powerful data acquisition and processing, to be considered as a viable sensor components. But, the real need of the market is the realisation of automated embedded systems which integrate biosensing components with microfluidics, data management hardware, and wireless communication devices. This is a key issue for nanotechnology, which can provide the decisive approaches as well as novel nanomaterials for the realisation of biosensing devices, which can tangibly reach the market and can be involved in commercial applications.

A PubMed search for reviews on the combination of "commercial" and "nanobiosensors" terms revealed 3 hits, in comparison with "commercial" and "biosensors" terms, that revealed 546 hits (accessed October 2012). This great discrepancy may be due to the lack behind the academic research to deal with arguments on the realisation of commercial nanobiosensors, or maybe nanobiosensors are not still being ready for the marketplace, while a number of biosensor devices have been already present on the market for many years. Despite this, it is assuming a broader position in the research community awareness to daily stimulates biosensor R&D based on nanotechnology, in order to realise biosensing systems able to compete with non-real time methodologies, and provide faster, less expensive and time-consuming systems. Crucial tasks are likely to take advantage of nanotechnology to provide technologies in the fields of new nanomaterials production, miniaturisation, communication, and energy storage, to make real the nanobiosensors industrial manufacturability.

6. Summary and conclusions

A search for articles on "glucose biosensors", performed on the main web browsers for scientific publications, like PubMed, Springer and Google Scholar, revealed thousands hits as reported in Fig. 4, indicating that data on the number of publications on glucose biosensors lagging behind academic research are extremely massive (accessed October 2012). However, the development of a sensing system for blood glucose poses several demands in the awareness of a commercial accomplishment. A successful biosensors is likely to be small, cheap and portable, to reach the interest of millions of diabetic patients which daily need to perform glucose test in a simple way and everywhere. In this context, a real challenge is to minimise blood volume and design an alternative system to avoid painful sampling. Very often, disposable single use analysis ensure to bypass contamination and reveal a simpler fabrication, even though an implantable biosensor integrated with microfluidics could provide a more accurate management, in term of glucose monitoring and insulin administration. From a manufacturing point of view, a biosensor for blood glucose monitoring need to be low in cost and adapted to a mass production. As a consequence, the biological components should display high storage and operational stability, and should enjoy a simple and stable calibration. Finally, all the required components, like biological recognition elements, transduction system, electronics and microfluidics, should be integrated to obtain an embedded system which ensure ease of use by the end-user.

In the aim of nanotechnology, biosensor technology has accelerated tremendously in terms of device complexity, usability and its ability to enter the commercial market, thanks to the application of new biocomposite nanomaterials (Zhang et al., 2009). In this context, nanotechnology accomplished significant efforts towards embedded sensing devices, by means of the generation of novel nanomaterial and nanostructures able to improve biosensing interfaces and develop intricate nanosystems. Indeed, inspired by nature, the synthesis of nanostructures has been proposed to assemble biomaterials, such as proteins, lipids, and nucleic acids, and enhance their unique functions (Siegrist et al., 2010).

In the last years, biosensor technology is taking advantage of novel materials, based not only on gene engineering, that has allowed to the generation of biorecognition molecules with desired features as well as synthetic molecules (aptamers, molecular imprinting polymers, biomimetics), but also on nanotechnology. Nanotechnology expects to have a significant impact on biosensor technology, allowing the construction of novel nanomaterials and, consequently, consenting miniaturisation, multi-sensor array set-up, and new immobilisation techniques. In this sense, nanotechnology, together with multi-disciplinary researches, provides emerging strategies in the development of new sensing systems, similar to point-of-care detection methodologies, able to monitor common disorders and to provide a balanced follow-up of several diseases, such as diabetes. In this context, this review presented recent advances in the application of nanomaterials for glucose biosensing.

7. Future perspectives

Despite the enormous potential of biosensors, commercially sensing devices seems to be available for a limited area of the market. In general, biosensors for biomedical analysis, and in particular for blood glucose monitoring, show several limitations, related to non-continuous monitoring, time response, and lifetime of the biological recognition element. Concerning nanobiosensors, even in this context there are several challenges to overcome, in the aim to obtain a commercial device.

Further characterisations need to be delivered, for example, on the mechanism of interaction between nanomaterials and biocomponents, as well as on the mechanisms governing the behaviour of nanocomposites on the surface of sensors, the processes to enhance signal to noise ratio and to increase signals transduction and amplification. Additional issues to evaluate for the commercialisation of biosensors in general and nanobiosensors in particular, involve the need to adapt the technology to the end-users skills, and elucidate them on the health risk/safety potentials of nanomaterials employed in the construction of nanodevices. On the other hand, the main obstacles for launching commercial biosensors are still linked to topics which withdraw from bio and nanotechnology matters. For example, blood pre-test sample processing could became necessary for nanobiosensors, compared with conventional biosensors.

Nevertheless, nanobiosensors, with respect to conventional biosensing systems, are revolutionizing biosensing technology, offering an attractive scenarios for the development of suitable hospital or home devices, with good linearity, precision, and resistance to common interferences. In the context of an implantable nanobiosensor for glucose monitoring, nanomaterials could help to improve biocompatibility and lifetime, minimise biofouling, decrease glucose transport time lag, and reduce cost of manufacture. Further aims should be devoted to the realisation of hand-held diagnostic tools capable of analysing multiple components and, consequently, to deliver a full screening, diagnosis, and management of diabetic patients state of health, monitoring several physiological metabolites in addition to glucose.

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