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Mini-review

Lectin-based biosensors as analytical tools for clinical oncology

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ABSTRACT

The review focus on the use of lectin-based biosensors in the oncology field, and ponders the potentialities of using these devices as analytical tools to monitor the levels of cancer glycobiomarkers in biological fluids, helping in the diagnosis, prognosis and treatment assessment.

Several examples of lectin-based biosensors directed for cancer biomarkers are described and discussed, and their potential application in the clinic is considered, taking into account their analytical features, advantages and performance in sample analysis.

Technical and practical aspects in the construction process, which are specific for lectin biosensors, are debated, as well as the requirements in sample collection and processing, and biosensor validation.

Today's challenges for real implementation of these devices in the clinic are presented, along with the future trends in the field.

1. Introduction

1.1. Definition and features of a biosensor

Biosensors are chemical sensors in which the recognition system is based on a biochemical or biological mechanism [1–3]. Recognition is the process of selective interaction between the biosensing element, which is immobilized on the sensor surface, and the analyte, present in the sample. The sensing element may be of biological origin or a synthetic material that biomimetizes a natural component.

The recognition event is characterized by two features: reversibility and selectivity [4]. The interaction is based on the establishment of noncovalent chemical bonds, such as ionic bonds, hydrogen bonds or van der Waals interactions, between the analyte and the sensing agent. The constant of this equilibrium reflects the affinity of the sensing element towards the analyte. If the biosensor response depends on the product concentration, then it will be affected by the analyte concentration in the sample [4]. Selectivity of the biosensor is the ability to respond preferentially to the presence of the analyte, and not to other sample components. Ideally, a biosensor should be specific (100% selective) for a particular analyte, so that the response would be exclusively correlated to the analyte concentration in the sample.

Different sensing elements may be used in biosensors, namely nucleic acids, enzymes or substrates, cells or biological tissues, and proteins or glycoproteins of diverse families (antibodies, lectins) that show affinity towards particular molecules (antigens, glycans), forming association complexes. In this case, the interaction could be more or less specific (for example, an antibody-antigen interaction is much more selective than a lectin-glycan interaction).

As for the transduction methods used in biosensors, they can be thermometric, optical, mechanical and electrochemical. Electrochemical-based methods are the most common transduction mechanisms employed in biosensors.

Since the final objective of a biosensor is to be commercialized and applied in real life, it should present some attractive features, namely: a) simple to operate, so it can be easily used; b) portable, to allow *in vivo* analysis; c) miniaturized, to reduce the amount of sample needed and to allow integration of several biosensors in arrays, increasing the throughput; d) free of sample preparation, to simplify the procedure and reduce assay time; e) disposable, to avoid cross-contamination between samples, repetitive calibrations and conditioning after each measurement; f) robust and with reproducible manufacturing, so that it is reliable and does not require calibration; g) sensitive and with low limits of detection, since the majority of biological analytes of interest are in very low concentrations in samples; h) highly selective, to avoid or minimize interference errors; i) quantitative, so that the biosensor response may be correlated to the analyte concentration or amount in the sample; j) compatible with established readout systems, which should be miniaturized as well, to enable the point-of-care (POC) or *in vivo* measurement; k) cheap, so that it can be not reusable without

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increasing the assay cost.

1.2. Advantages of biosensors in point-of-care analysis and precision medicine

Among the several uses of biosensors in real life context, healthcare applications are one of the most significant. Biosensors can be applied for *in vitro* or *in vivo* determinations of particular analytes with physiological or pathological relevance [5–15], in particular biomarkers of physiological or pathological states (in cancer, cardiovascular diseases and hormone-related health problems) [6,8,11,13,16–19].

The developments in biosensor research allow presenting, at this moment, assays with short and separation-free procedures, with the possibility of performing the measurement *in situ*, which has advantages with respect to common enzyme-linked immunosorbent assays (ELISA) [20,21]. Also, the possibility to construct label-free biosensors increases simplicity in the biosensing process and enables to reduce the assay time, which is advantageous for POC analysis [22,23].

The possibility of miniaturization of the biosensing devices allows, on one hand, to decentralize the analysis and, on the other hand, to work in the micro-scale, reducing the volume of reagents and samples required to perform the analysis. This makes the operation cheaper (allowing mass production) and less hazardous, when compared to the macro and semimicro-scale techniques [24]. Furthermore, the automation allowed by the use of biosensors enables screening of populations in a simple and fast way. Also, there is the option to immobilize more than one biorecognition agent on the biosensor, creating thus a multi-analyte biosensor that may be employed to simultaneously determine a series of analytes. This may have advantages in the profiling of samples, since in many pathological situations a profile of markers provides more accurate information than the monitorization of a single marker [7,25–30].

The use of biosensors in clinical testing is more practical, faster, more user-friendly, less expensive and less technically demanding than microarray or proteomic analysis [7]. The later approaches are not practical for routine testing due to their complexity and cumbersome and lengthy protocols, which may affect reproducibility and must be performed by experienced technicians.

Nowadays, precision medicine centred in the patient can make the difference in the life quality of patients, by proposing screenings, diagnosing entire families for particular mutations before the disease reveals and offering treatments with more quality (higher efficacy and less adverse effects) by adjusting doses to the patient metabolism. In all these actions, analytical tests based in biosensors may be employed, which selectively detect particular biomarkers that are indicative of the disease presence and progression, and treatment efficacy [31].

2. Application of biosensors in clinical oncology

Cancer incidence is increasing and will continue to increase in the next decades, due to the raise in life expectancy of populations and the difficulty to control some of the stigmas that characterize today's societies, such as sedentarism and obesity [32,33]. This is, obviously, a worrying scenario, and the need to intensify prevention is mandatory. Furthermore, a personalized prevention is becoming a target, which will allow to improve the individual adhesion and the efficacy of interventions [34].

Both in population screening methods and patient stratification, the use of biosensors for biomarkers is, theoretically, an useful option [28,35,36]. Detection of serum cancer biomarkers would permit to carry out screenings in a fast way, helping on the precise diagnosis in an early phase, and this could be performed by using biosensors towards the cancer-associated biomolecules produced and secreted by tumour cells. Unfortunately, the success of cancer biomarkers in screenings has been disappointing, mainly due to insufficient sensitivity. Biomarker amounts secreted by tumour cells are very low and the dilution effect of blood decreases even more its concentration. A solution to this may be to use proximal fluids [36]. In addition, many cancer biomarkers are elevated in benign diseases [28,37], which require further testing to confirm malignancy.

In advanced or metastatic disease, current guidelines recommend the assessment of response to treatment through history, physical-clinical examination, radiological exam and routine blood tests [38]. Among them, tumour biomarkers are quantified, namely CA15-3 and CA27-29 for breast cancer, α-fetoprotein for liver cancer, PSA for prostate cancer, CA125 for ovarian cancer, CA19-9 for pancreatic and ovarian cancer, CEA for colon, gastric, pancreatic, lung and breast cancer, HER2 for breast cancer and hCG for testicular and ovarian cancer [8,37,39]. Although a change in a tumour biomarker alone is not sufficient for treatment decisions, its level is considered helpful in monitoring the response and for diagnosis of distant metastasis [8,37,40]. The use of serum cancer biomarkers in therapy monitoring may reduce the follow-up expenses by up to 50% as compared to the use of imaging techniques [41].

3. Lectin-based biosensors as potentially useful tools in clinical oncology

3.1. Aberrant glycans as cancer biomarkers

Aberrant glycosylation of sphingolipids and proteins is a feature of essentially all types of experimental and human cancers. According to the current knowledge, this is a result of the oncogenic transformation of cells, which induces invasion and metastasis [42,43].

Cancer-associated abnormal glycosylation includes incomplete synthesis of *O*-glycans (namely the formation of sialyl-Tn, Tn and T epitopes), altered glycosylation of mucins (with changes in MUC2 and MUC5AC for colon cancer, MUC1 in several types of cancer and CA125 in ovarian cancer), alterations in the synthesis of histo-blood group-related antigens (A and B glycan epitopes, which are reduced or absent, being replaced by the precursor H epitope, and Lewis antigens, whose profiles are different from that seen in adjacent normal tissues), increased (β1,6) branching of *N*-linked glycans, namely in breast and colon cancers, alterations in sialylation and fucosylation (for the Lewis family antigens and T and Tn antigens), and synthesis of HPA-binding glycans [43–46].

These aberrant epitopes have been identified as tumour-associated antigens and, clinically, their expression is inversely correlated with survival of patients [43], being more obvious in early stages than in later stages of human cancer [47], when other factors may be involved. The antigens may, therefore, be useful as prognostic markers [44,45].

The use of glycan biomarkers instead of protein biomarkers for cancer has several advantages: (1) glycan biosynthesis is more susceptible to be affected by disease than protein biosynthesis, because glycans are metabolic products. Small changes in proteins cause amplified variations in glycans; (2) glycans are synthesized only in the Golgi apparatus or in the endoplasmic reticulum, therefore abnormal glycosylation can potentially impact all glycoproteins produced in the tumour cell; (3) with the current analytical techniques, it is easier to measure oligosaccharide expression than protein expression [48,49]; (4) one protein can carry many copies of an altered glycan, which may also be added to other scaffolds, generating an amplification effect; (5) glycosylation acts to shield the peptide backbone from proteolytic degradation, thus glycan-based biomarkers are, in theory, more stable than unmodified proteins, in a disease setting [50]. Further, glycans are secreted by the cells and are abundant in most biological fluids, such as serum, plasma, saliva and tears. The glycan may be used as a marker by detecting its amount or, in a more deep analysis, by detecting site-specific glycosylation [49]. In conclusion, capitalizing on alterations in cancer-related protein glycoforms enables an increase in diagnostic sensitivity and specificity [50]. For example, the employment of the lectin from *Maackia amurensis* with different affinity for PSA glycoforms

Table 1
Glycoprotein cancer biomarkers approved by FDA and currently used in clinical practice.

Tumour marker	Cancer type	Year of FDA approval	Detection type	Clinical application
α-1 acid glycoprotein (AGP)	breast	2009	glycoprotein concentration	predictive
α-fetoprotein (AFP)	germ-cell liver	1992/2008	protein concentration and core fucosylation (for AFP-L3)	diagnosis staging differential diagnosis of NSGCT detecting recurrence monitoring therapy
cancer antigen 125 (CA125)/MUC16	ovarian	1997/2009/2011	protein concentration	prognosis detecting recurrence monitoring therapy
cancer antigen 15-3 (CA15-3)/MUC1	breast	1997	sialylated *O*-linked oligosaccharide on MUC1	monitoring therapy
cancer antigen 27–29 (CA27-29)	breast	1997/2002	MUC1 protein level	monitoring therapy
carbohydrate antigen 19-9 (CA19-9)	pancreatic ovarian	2002	SLe[a] on mucin glycoproteins and gangliosides	monitoring disease status monitoring therapy
carcinoembryonic antigen (CEA)	colon gastric pancreatic lung breast	1985	protein concentration	prognosis detecting recurrence screening for hepatic metastases monitoring therapy
estrogen receptor (ER) and progesterone receptor (PgR)	breast	1999	formalin-fixed paraffin-embedded tissue	select patients for endocrine therapy prognosis response to therapy
human epidermal growth factor receptor 2 (HER2/neu)	breast	1998	protein concentration	prognosis select patients for trastuzumab therapy
human epididymis protein 4 (HE4)	ovarian	2008/2011	protein concentration	prognosis detecting recurrence monitoring therapy
Lewis *a* antigen (Le[a])	ovarian	2002	carbohydrate concentration	prognosis monitoring therapy
prostate-specific antigen (PSA)	prostate	1986/1994/2012	protein concentration	screening (with DRE) diagnosis (with DRE) discrimination cancer from benign disease
thyroglobulin (Tg)	thyroid	1997	protein concentration	prognosis monitoring therapy
transferrin	all cancers blood cancer	2009	glycoprotein concentration component of MHC I molecule (blood cancer)	predictive

NSGCT – non-seminomatous germ cell tumours; MUC – mucin; DRE – digital rectal exam.

allows to distinguish between blood samples from individuals with benign prostatic hypertrophy and prostate cancer patients [51], whereas standard PSA tests can't [52]. Nonetheless, it is curious to observe that very few cancer biomarker discovery strategies have been designed to focus on aberrant glycosylation, which can be due to the complexity and heterogeneity of carbohydrate structures carried by glycoproteins, which have made it difficult to understand their functions [50], as well as the lack of tools to synthesize and analyze them accurately [53].

In spite of the great plethora of cancer biomarkers discovered so far, very few have been approved by FDA for clinical use. Table 1 depicts the ones that are glycoproteins [39,54–56]. Although the majority of cancer biomarkers currently used are glycoproteins, which are structurally altered in their glycan moieties, only α-fetoprotein, CA15-3 and CA19-9 are clinically monitored for their glycan changes, while the other cancer biomarkers are monitored for their total protein levels [57]. Considering the case of α-fetoprotein, the aberrantly fucosylated glycoform produced by liver was approved by FDA as a biomarker for hepatocellular carcinoma. The commercial assay is based on a lectin-antibody sandwich design, using *Lens culinaris* agglutinin (LCA) to assess the percentage of fucosylated glycoform. This example demonstrates the clinical utility of cancer glycobiomarkers [53].

In all FDA-approved cancer glycobiomarkers, the detection methodology is an immunoassay, which means that they are detected through the use of antibodies. Nonetheless, according to their molecular structure, they could be also detected by lectins, considering the glycan as the analyte [50,55].

For the approved glycoprotein biomarkers, the majority cannot be employed for diagnosis, since they can only detect late stage disease and/or cannot distinguish between cancer and benign disease. All of them show some unspecificity and may give false positives. Nevertheless, they can be used for staging, detecting recurrence and monitoring treatment.

3.2. Lectins as biosensing agents in biosensors for glycan biomarkers

Biosensors devoted to detect cancer-associated glycan antigens are, frequently, based on lectins, which act as the biosensing agent [55,58]. Lectins have been used for cancer biomarker discovery research and have been employed in diverse applications in cytochemistry, histochemistry and immunohistochemistry for the detection and characterization of glycoconjugates in human and animal cells and tissue surfaces [59–61], but they have found more applications in the sensor field [23,62].

Lectins are proteins or glycoproteins that selectively bind to particular glycan structures, through hydrogen bonds, metal coordination, van der Waals and hydrophobic interactions, not modifying the carbohydrates to which they bind. The carbohydrate-binding site in the lectin is located within the carbohydrate-recognition domain (CRD). Lectin binding sites consist of a primary binding site which is capable of recognizing in a specific way a single monosaccharide residue. Often, there are additional subsites that can be occupied by sugar residues connected to the one bound at the primary site. In subsite binding, one monosaccharide (as a rule the terminal one) binds to the primary binding site of the lectin and then other monosaccharides in the chain bind to secondary subsites. This causes a slight increase in affinity in a

Table 2
Cancer glycobiomarkers with their aberrant glycosylation and the potential lectins that can be used for their biosensing.

Tumour marker	Cancer type	Type of biomarker	Glycan modification	Lectin	Ref.
AFP	liver	glycoprotein	fucosylation	*Lens culinaris* agglutinin	[68]
				Phaseolus vulgaris erythroagglutinin	[69]
CA125/MUC16	ovarian	glycoprotein (mucin)	truncated Tn (O-linked), STn, core fucosylation	*Glycine max* agglutinin	[70]
				Vicia villosa agglutinin	[71]
CA15-3/MUC1 CA27-29	breast ovarian lung pancreatic prostate	glycoprotein (mucin)	truncated Tn, STn, 3-sulfated core 1, terminal sialylation	galectin 4	[72]
CEA	colorectal	glycoprotein	↑ Le^x, Le^y, high mannan structures, ↓ core fucosylation, ↑ branching, ↑ Neu5Ac	*Phaseolus vulgaris* agglutinin wheat germ agglutinin	[73]
HER2/neu	breast	glycoprotein	proximal fucosylation	concanavalin A wheat germ agglutinin	[74]
HE4	ovarian	glycoprotein	blood group antigens	*Griffonia simplicifolia* agglutinin	[55]
PSA	prostate	glycoprotein	↑ α-2,3 Neu5Ac, ↑ α-1,2 Fuc, β-GalNAc	*Maackia amurensis* agglutinin	[51]
				Sambucus nigra agglutinin	[75]
Tg	thyroid	glycoprotein	terminal galactosylation, asialylation	*Lens culinaris* agglutinin	[76]
				Wisteria floribunda agglutinin	[77]

Le^x – Lewis x antigen; Le^y – Lewis y antigen; Neu5Ac – neuraminic acid; Fuc – fucose; GalNAC – N-acetyl-galactosamine.

factor of 2–50 [63].

The lectin-glycan interaction is generally weak, in the millimolar to micromolar range, but multiple interactions can be established between both elements to enhance their binding. Several lectins have the ability to establish more than one separate connection of the same kind with other glycans through ligand-receptor interactions, which is known as multivalency. Multivalency in lectins allows achieving very tight binding to the glycan (with a higher association constant) [64], and selectivity towards a particular target is increased by several orders of magnitude [65,66].

A great diversity of glycan structures can be detected by an equally varied lectin collection. In addition, many lectins have become commercially available in high purity. These two factors have prompted the use of lectins in biosensors for glycan biomarkers. Compared to antibodies, lectins are less expensive, easier to be obtained and present longer shelf life [25].

Table 2 shows a list of cancer glycobiomarkers with their aberrant glycosylation, and examples of lectins that can be used for their biosensing [23,55]. The table is not exhaustive and for each biomarker only one or two examples of lectins are indicated. For some of the biomarkers, other lectins have been used for detection [67].

Different designs can be used to construct a lectin biosensor: the lectin can be immobilized at the device surface by different binding strategies, for example through self-assembled monolayers, and allowed to bind to glycans, free or in glycoconjugates (Fig. 1A), or a sandwich assay can be designed, where an antibody or a lectin is immobilized on the sensor surface, then the sample is incubated and the analyte binds to the sensing element and, after, a lectin or an antibody, which can be labelled, binds to the analyte so that the binding event can be measured (Fig. 1B and C). The sandwich assay can also be performed with two lectins instead of a lectin and an antibody. In the first situation (Fig. 1A), no labelling is required and the complex formation is detected impedimetrically [78].

3.2.1. Lectin-based biosensors for cancer biomarkers

Several lectin-based biosensors have been developed for a variety of cancer biomarkers and biosensors for truncated *O*-glycans are among the more recurrent ones. An *Arachis hypogaea* lectin (PNA) biosensor was developed for the detection of T antigen [79]. According to the authors, this was the first description of nanoparticle-based biosensing of glycan markers of diseases. The protocol relied on the competition between a nanocrystal (CdS)-tagged sugar and the target sugar for the binding sites of surface-confined lectin, monitoring the extent of competition through highly sensitive electrochemical detection of the captured nanocrystal. This development was expected to allow decentralized detection of carbohydrate moieties and lectin-carbohydrate interactions. Although the protocol exhibited excellent discrimination between target and nontarget sugars and the trend in sensitivity was according to what was expected for PNA, samples were not analyzed, so the clinical utility could not be demonstrated.

The same group developed other biosensors containing PNA and *Sambucus nigra* lectin (SNA) for the detection of glycoconjugates carrying the T and STn epitopes, respectively [80]. Electrochemical impedance spectroscopy (EIS) was employed as the transduction method. The proposed biosensors proved to be effective in the detection of glycan-lectin interactions in a label-free fashion, with potential applications in the detection of cancer biomarkers, and the technique was shown to be fast and sensitive. Several glycoconjugates and glycoproteins carrying the T and STn glycans were analyzed and selective responses were obtained for both lectins, with excellent limits of detection. Unfortunately, their behaviour in the analysis of real samples was not evaluated.

Another impedimetric SNA-I biosensor was developed and used to detect the STn glycan in the glycoproteins fetuin and asialofetuin [81]. On polycrystalline gold modified by an aminoalkanethiol linker layer, gold nanoparticles were attached and, then, SNA was covalently immobilized on a mixed self-assembled monolayer (SAM) formed on gold nanoparticles. Finally, the biosensor surface was blocked by poly (vinyl alcohol). Lectin microarray assays were performed to validate the results obtained with the biosensor. Analytes could be detected down to attomolar levels and the biosensor was able to provide information about the amount of glycan determinants present on a particular glycoprotein. Once again, no real samples were analyzed, in spite of the improved analytical features of the biosensor.

SNA-I and *Vicia villosa* lectin (VVA) impedimetric biosensors were developed for the detection of STn and Tn antigens, respectively, in serum samples [82,83]. The work demonstrated the feasibility of employing SNA-I and VVA to selectively recognize the STn and Tn epitopes in glycoproteins and the constructed biosensors were effective in the analysis of serum samples with the ability to discriminate in a fast way between cancer and healthy status. However, in order to confirm the discriminative power of the develop biosensors, a more extensive validation procedure must be carried out, through the analysis of a larger set of samples and data processing, according to official guidelines.

A SPR biosensor using PNA was develop for the detection of T antigen, at concentrations down to 50 nM with no significant interference of a closely-related disaccharide [84]. In this case, the lectin was not directly immobilized at the device surface. Instead, an anti-PNA

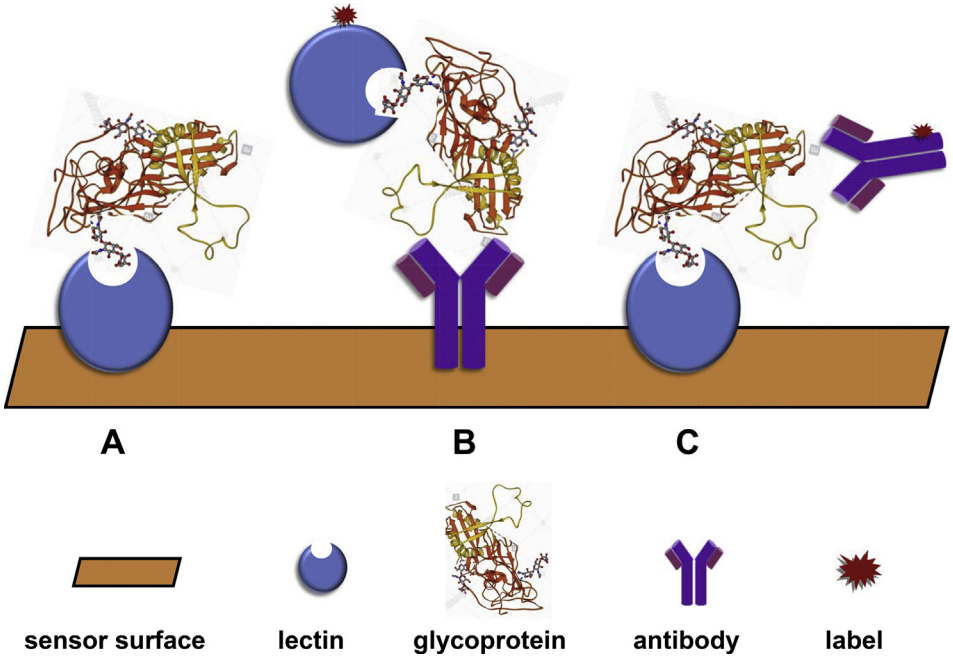

Fig. 1. Configuration of lectin-based biosensors with direct (A) or sandwich immobilization protocol (B and C). The sensing element (lectin or antibody) can be immobilized through different strategies that include covalent and non-covalent binding. The binding of the glycoconjugate in the direct protocol is usually carried out impedimetrically and no labeling is required. For the sandwich protocol, the lectin or antibody is labelled with an enzyme or fluorescent molecule in order to allow the measurement of the binding event. Note: for the sandwich C-scheme, another lectin can bind to the glycoprotein, instead of an antibody.

antibody was fixed and, after, PNA was allowed to bind to the antibody. Finally, when the T antigen was incubated, lectin-glycan interactions could be established. A notorious advantage of this biosensor was its ability to be fully regenerated by incubation of PBS, pH = 1, for 1–2 min, and thorough rinsing.

An ultrasensitive diagnostic miniature platform called "NanoMonitor" was developed for glycan biomarker detection, allowing glycoprofiling in a multiplex format [85]. The surface of a silicon chip with an array of gold electrodes was modified with a nanoporous alumina membrane on the top of each electrode. Using SNA and *Maackia amurensis* agglutinin (MAA), immobilized on the electrodes surface, subtle changes in glycosylation of fetuin and human pancreatic cancer cell line BXPC-3 were identified, being measured by EIS.

Biosensors for cancer biomarkers which are glycoproteins have also been reported. Yang and co-workers functionalized single-wall nanotubes (SWNTs) adsorbed onto an electrode with wheat germ lectin (WGA), *Lens culinaris* lectin (LCA), *Canavalia ensiformis* lectin (Con A), SNA and *Datura stramonium* lectin (DSA) to detect α-fetoprotein in serum samples, through EIS [86]. It was found that the employment of SWNTs as immobilization platform could reduce the background and enhance the EIS response. In addition, the employment of the five lectins allowed to evaluate the *N*-glycan expression of α-fetoprotein. Serum samples of patients with liver cancer were analyzed and the developed biosensors were able to discriminate α-fetoprotein between healthy individuals and cancer patients. Although the results show potential utility of the biosensors, an extensive validation through analysis of larger sets of samples is required.

CEA was detected together with a drug to cure colorectal cancer, cetuximab, using a well-designed strategy employing square-wave voltammetry [87]. The approach was based on the displacement of quantum dot (QD)-labelled glycans by the target glycans using two lectins, Con A and *Euonymus europaeus* lectin (EEL), as the recognition elements. Upon incubation with the sample containing both the drug and the biomarker, QD conjugates were displaced from the surface and released Zn^{2+} and Cd^{2+} ions were analyzed by stripping voltammetry. The reported biosensor, used for the simultaneous detection of the biomarker and the therapeutic drug, is an innovative approach and is expected to be developed as a potential technique for quantitative pharmacology.

A different biosensor was designed for the detection of CEA, using the lectins WGA, Con A and LCA [88]. The chronoamperometric biosensor was constructed by covalently coupling the lectins on the surface of a gold nanoparticle-cysteamine-modified electrode. A lectin-target-antibody sandwich-type conjugate was formed when the biosensor was successively reacted with target protein and horseradish peroxidase labelled anti-CEA antibody probe. The lectin-based biosensor array was successfully applied to evaluate the *N*-glycan expression of CEA and discriminate CEA between serum samples of healthy individuals and liver cancer patients. The results showed the feasibility of the developed device to be applied in cancer detection and further pathological study of carcinogenesis.

A SPR lectin biosensor was proposed for the detection of hyperglycosylated human chorionic gonadotropin (hCG) produced by malignant gestational trophoblastic neoplasias and male germ cell tumours [89]. The sandwich-type immunoassay utilized carbohydrate-free antibody fragments covalently attached to the chip, followed by addition of control or hCG-containing samples and then individual lectins. The biosensor was able to compare the glycosylation patterns of hCG among urine specimens from individuals with a normal pregnancy, invasive mole, choriocarcinoma and male germ cell tumours, using the antibody fragments as capture reagents and a panel of eight lectins, five recognizing neutral sugars and three recognizing sialic acid. The authors claimed that, by using deglycosylated antibody fragments as capture reagents, the clinical potential of lectin discrimination of condition-specific glycoproteins can be explored.

A biosensor for CA15-3 was designed by using a fluoro-microbead guiding chip (FMGC) and the lectins SNA and PNA conjugated with fluoro-microbeads [90]. The detection was performed through an antibody-lectin sandwich assay, as follows: a capture antibody against CA15-3 that binds to the peptide backbone of CA15-3 was immobilized on the gold-patterned sensing surface. Then, the immunosensor was incubated with CA15-3 and finally the lectin-conjugated fluoro-microbeads were put in contact with the sensor. The fluoro-microbeads biospecifically bound on the FMGC were detected and recorded using a fluorescence microscope. The authors concluded that the PNA- and SNA-based assay system exhibited decreased detection limits comparing to the conventional ELISA, while covering the clinically required determination range of target analyte. Furthermore, it was demonstrated that employing a lectin-antibody reaction pair could be a promising alternative to the typical antibody-antibody sandwich assay.

Nevertheless, the biosensor was not validated through the analysis of real samples, to definitely conclude about its usefulness in the diagnosis of breast cancer and its recurrence-metastasis.

A lectin-based immunosensor was developed for the detection of PSA using three lectins with different carbohydrate specificities: SNA-I, specific for α-(2,6)-terminal sialic acid, *Lotus tetragonolobus* agglutinin (LTA), recognizing α-L-Fuc and MAA-II, recognizing α-(2,3)-terminal sialic acid [75]. The anti-PSA antibody was covalently immobilized on a SAM formed on a gold disk electrode and then the biosensor interface was exposed to PSA. After, the three lectins were used to glycoprofile PSA glycans to complete a sandwich biosensor configuration. The analysis of sialic acid by the lectin-based immunosensor was shorter and simpler than the analysis performed by mass spectrometry (MS), and showed to have potential to be applied in glycoprofiling of PSA. The authors pointed the advantages of the developed biosensor, namely the ability to detect increasing/decreasing amounts of α-(2,6)-Neu5Ac or α-(2,3)-Neu5Ac on the selected biomarker (even tri- and tetra-antennary glycans, which can be present on PSA), since lectin biosensor could detect glycans in a quantitative way, high sensitivity with a low limit of detection and small sample consumption. The main drawback of the proposed biosensor was the low throughput of analysis, which could be addressed by working in an array format of analysis.

Although biosensors do not provide detailed and complete structural information about glycosylation as MS and other analytical techniques frequently used in glycomics do, they offer quantitative information, together with the selectivity given by the lectin, which may be sufficient for high-throughput analysis in diagnostic and/or screening assays [91].

4. Technical and practical aspects in the construction process

4.1. Lectin immobilization

Immobilization of the biorecognition element in a biosensor is of upmost importance because the immobilized molecule should conserve its activity intact as it would be in a free state. Therefore, the immobilization procedure impacts on the biosensor performance.

Biological recognition agents used in biosensors can be immobilized in a thin layer at the transducer surface by using different processes, namely entrapment behind a membrane, entrapment within a polymeric matrix, entrapment by SAMs or bilayer lipid membranes (BLMs), covalent binding on membranes or surfaces activated through bifunctional groups or spacers and bulk modification of the electrode material [1]. Adsorption methods are reversible, simple and fast but the binding is weaker, the orientation of the biomolecule may be unfavourable and its functionality and stability may be impaired [92]. Also, adsorbed biomaterials are more prone to changes in the surrounding environment (changes in pH, temperature and ionic strength) [23]. Covalent immobilization procedures are more complex than entrapment modes, but are especially adequate when the sensor is very small so that the membrane must be fabricated directly on the transducer surface. In this method, the biorecognition element shows more stable and reproducible activity [1].

The most frequent surfaces used for biosensor construction are metals (gold, silver, platinum), natural hydroxylic polymers, diverse carbonaceous materials (graphene, glassy carbon), glass and silica [93]. Recently, the application of screen-printed electrodes has attracted an increasing interest due to their suitable characteristics, such as simple and low-cost fabrication, mass production, and conveniently practical application [94–97]. Also, engineered materials such as nanoparticles, nanotubes and QDs have been applied in conjunction with lectins to design novel biosensors with improved analytical characteristics (see section 3.2.1. for examples).

For the particular case of lectin-based biosensors, one of the more frequent immobilization methods used is entrapment by SAMs made of alkanethiols with different terminal groups (−SH, $-NH_2$ or −COOH) [23]. SAMs are organic assemblies spontaneously formed by strong adsorption on a solid surface such as gold, silver or platinum [98]. The attachment of the alkanethiol to the lectin is performed by amine coupling, at the free carboxylic terminal of the alkanethiol. The covalent binding is carried out via activation agents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride or N,N′-dicyclohexylcarbodiimide and N-hydroxysulfosuccinimide [92]. More details on immobilization techniques can be found elsewhere [23].

4.2. Detection methods

Lectin-based biosensors employ different transduction methods, mainly electrochemical (impedimetric, voltammetric and chronoamperometric) and optical (SPR and fluorescence). For direct assays, impedimetric detection is the most common. Impedimetric detection has the advantages of easy miniaturization and label-free operation (attractive for POC analysis), that is, unlabeled targets may be detected by monitoring changes in surface impedance when the target molecule binds to the immobilized recognition probe. Furthermore, EIS applies small voltages which do not damage the biomolecule probe layer, in opposition to voltammetry or amperometry, where more extreme voltages are applied [22]. Impedance lectin biosensors are versatile because they can detect a variety of different glycan molecules, by simply changing the biorecognition lectin. Label-free operation means a simpler measurement procedure because the labelling step is omitted and allows detection of the analyte in real time, which is usually not possible with labelled systems [99]. In addition, it has been observed that the labelling process may affect the bioaffinity between the probe and its target, which can lead to false positives or negatives [78,81].

For sandwich assays, the second biorecognition element must be labelled for measurement (Fig. 1), by fluorescence or other optical method. Labelled designs result in enhanced selectivity and sensitivity, since a second biorecognition element is used and the label greatly changes the impedance [22]. In sandwich assays, the affinity and cross-reactivity of the antibody must be considered, since they influence the biosensor selectivity. Monoclonal antibodies are highly specific to one epitope on an antigen but are more expensive to produce, compared to polyclonal antibodies.

EIS detection is gaining popularity in lectin-carbohydrate studies [23,78] and some disadvantages that have been pointed out, namely the higher cost compared to other electrochemical techniques and a complex data analysis, are not problematic at the present time. Anyway, the choice of the biosensor design must be driven by the particular application.

4.3. Particularities on the use of lectins

Lectins are obtained from a great diversity of living organisms, so an extraction procedure must be carried out, followed by a purification step, before they can be used. Nowadays, a wide diversity of commercial lectins is available in high purity, which avoids the extraction and purification work. If the choice is using a commercial lectin, the provider must be chosen carefully, by evaluating the analytical performance of the same lectin provided by different suppliers. Some lectins have isoforms with different affinity patterns, so the most adequate isoform must be selected cautiously, and we must ensure that it is provided in a pure form and not in a mixture of isoforms, which can affect the biosensor performance [80]. Also, the binding behaviour for the same lectin may change according to the supplier [53,80,100]. All the work should be carried out using the lectin from a unique supplier to avoid variability which can affect the biosensor reproducibility.

If the choice is to extract and purify the lectin from a natural source, the same procedures must be systematically followed, starting from the cultivation of the natural source (if possible), to assure reproducibility in the final product. Using the same reagents to prepare the biosensors is essential to allow comparison of results and to guaranty

reproducibility in the biosensor fabrication and performance. For lectin purification, affinity chromatography and high performance liquid chromatography (HPLC) are the techniques commonly used. Isolation and purification methods used for lectin purification have been reviewed [101].

Another important aspect is the lectin stability. Usually, lectins are stored at low temperatures (−20 °C) before use, to maintain their binding capacity. After the biosensor is constructed, stability assays must be performed at different temperatures, to assess the best storage conditions of the device, keeping in mind the selective binding ability of the lectin. In several papers, storage conditions of the biosensors consist of low temperatures (4 °C) at particular pH (usually employing buffer solutions as the solvent). Frequently, the lectin optimum pH matches the optimum pH for samples, which simplifies the analytical process.

Many lectins require divalent cations to bind to carbohydrates. The divalent ions Ca^{2+}, Mn^{2+}, Mg^{2+} and Zn^{2+} are involved in carbohydrate recognition either indirectly, by shaping the combining site, or through direct binding of the carbohydrate to Ca^{2+}. In some lectin groups, Ca^{2+} helps to stabilize the lectin domain, organizes the site for ligand binding and participates in subunit oligomerization. This divalent cation also plays a structural role and helps neutralizing repulsive forces between anionic charges in ligand and lectin [102]. Some lectins require one, two or three of these ions and others function without the presence of any. Hence, before starting the work, the requirements of a particular lectin in terms of divalent cations should be revised. If needed, they can be included in the buffer solution used throughout the work.

Lectins are not as specific as antibodies and one lectin may bind to more than one glycan structure. This results in some unspecific binding and a not so sharp response of a lectin-based biosensor. This problem is aggravated when the biosensor is meant to be used in the analysis of complex samples, where a huge diversity of glycan structures may be present. This type of unspecific interaction cannot be eliminated since it depends on the selectivity of each lectin, so a certain degree of unspecificity towards non-target glycans is always expected in lectin-based biosensors. A possible way to circumvert this limitation could be the integration of an aptamer or antibody binding agent in the lectin biosensor, which could improve the biosensor selectivity [58]. During the development of a new biosensor, selectivity and cross-reactivity assays must be performed, by analyzing different glycans or glycoproteins containing the glycan epitope of interest and others, to fully characterize the biosensor selective behaviour. In some cases, samples need to be partially purified or somehow its complexity must be reduced, without losing the analyte, to minimize unspecific interactions with the lectin.

Regeneration of biosensors is an important issue, because if regeneration is possible, the costs with the analysis could be significantly reduced, since the same biosensor could be reused a number of times without the need for constant recalibration. In practice, it is very difficult to regenerate biosensors because the interaction between the biorecognition element and the analyte is strong or very strong, as is the case of the interaction antigen-antibody. As the affinity constant is generally very large, such systems are irreversible (single-use biosensors) or regenerated by dissociation of complexes by chaotropic agents like glycine-HCl buffer at acidic pH, Tris-NaCl at pH 8.5 followed by sodium acetate-NaCl at pH 4.5 or Tris-NaCl at pH 3.0, for different lectins [103,104]. The process used for regeneration must assure that there is no significant loss of activity of the biorecognition agent or loss of reproducibility of measurements. Whereas in some cases up to 20 incubation-regeneration cycles could be performed without significant loss of binding capacity of lectins [104], in other applications the use of glycine buffer at pH 2.5 caused a decrease in an immunosensor stability around 40%, indicating degradation of the recognition layer [103].

5. Sample analysis

5.1. Sample type

Both tissue-based and blood-based cancer glycobiomarkers have the potential to reveal important information on the disease state. Nevertheless, gathering blood samples is easier in terms of technical procedures, besides being less invasive than collecting tissue samples and allows to obtain larger sample amounts. In addition, blood glycoproteome is the most complete and representative, containing other tissue glycoproteomes as subsets, namely the aberrant glycoproteins secreted by tumour cells into the blood stream, which can be detected almost immediately after secretion, within a time scale of minutes to hours depending on the site of origin [105,106].

However, the analysis of blood samples for cancer glycobiomarkers has its own difficulties as well. Albumin corresponds to 55% of all the blood proteins and the 20 most abundant proteins correspond to 99% of all protein mass [105]. The dynamic range comprises 10 orders of magnitude [105]. Thus, the detection of low-abundant cancer glycobiomarkers, immersed in a very complex biological glycoproteome, is nearly impossible without the aid of biochemical techniques that reduce sample complexity.

The detection of cancer glycobiomarkers using biosensors is mainly performed through the analysis of blood serum since its composition is less complex than that of plasma [107]. Serum is the solution obtained after whole blood or plasma is allowed to clot, and has a different biochemical composition because fibrinogen is removed, as well as other proteins [108]. The protein concentration of serum is lower than that of plasma, which is largely due to the removal of fibrinogen [109].

5.2. Collection and storage aspects

To reduce variability in results attributable to biological variation and not related to the disease, some conditions must be considered when collecting blood samples. First, the health status of the subjects must be known (both for cancer patients and for control healthy individuals). Second, pre-defined exclusion criteria must be applied (for example, patients with concomitant chronic diseases that may affect the serum concentration of glycobiomarkers), introducing variability that may be erroneously attributed to cancer, should not be used, unless the study includes the analysis of patients with those characteristics. Third, physiological features (namely age, gender, lifestyle and geographical region) must be, as much as possible, similar for the control set and for the cancer cases set, to avoid bias in the results. Fourth, pre-analytical phase must be standardized including subject preparation (fasting, exercise, and posture), sample collection and storage until the analytical phase is carried out. Many blood biomolecules including proteins increase their concentration on venous stasis. Clotting conditions, transport time and temperature and centrifugation temperature, as well as the number of freeze/thaw/freeze cycles affect serum composition and stability, therefore influencing the results [107]. In many proteomic studies it has been demonstrated that all the mentioned factors may cause variation in the blood concentration of biomarkers [110,111]. The only way to annul that is to follow rigorously the same procedures to collect, transport and store all samples. Fifth, analytical variability during the testing process should be minimized and the data obtained must be statistically evaluated and dissected into analytical, within-subject and between-subject components, by using adequate statistical methods [112]. Processing and analysis of all samples from control and case sets must be carried out with the same procedures. Controls and cases should be collected in the same population, region or institution, to avoid bias due to one of these factors and not accountable to the disease. Other sources of bias can be operator-dependant, instrument-dependant and/or reagent lot-dependant. Sixth, frequently the analysis is to be performed some time after collection. Therefore, storage conditions must ensure glycobiomarkers stability. One of the difficulties in

sample analysis is the lack of adequately preserved biological specimens. The establishment of biobanks by research and clinical institutions is essential to gather sample collections, with statistically significant sizes to be used in the validation of biosensors.

Samples should be randomized and blinded before analysis to avoid bias.

5.3. Why processing?

Due to the wide dynamic range of protein and glycoprotein concentrations in serum (10^8 or larger [113]), the detection of cancer glycobiomarkers can be done only if serum is previously fractionated, to remove or, at least, substantially reduce the most abundant elements. The use of separation and fractionation techniques to look deeper into the serum glycoproteome parallels the development of the separations themselves. Currently, two-dimensional gel electrophoresis and liquid chromatography are separation techniques that allow to separate glycoproteins according to their isoelectric point, molecular mass and polarity [114,115].

Nonetheless, the huge complexity of the glycoproteome is not completely resolved using these techniques (their dynamic ranges are typically 10^2-10^4 [105]), so other fractionation methodologies must be employed, namely immunoaffinity subtraction, that can be performed using antibodies (immunoprecipitation) [116], lectins (lectin affinity chromatography) [61] or other ligands that selectively bind to the diverse glycoproteins in serum. Depletion strategies may present, however, some complications. First, co-depletion of low-abundant proteins and glycoproteins may occur, along with the high-abundant ones [117]. Second, albumin itself carries other proteins and glycoproteins in serum, so by removing albumin, these bound proteins may also be depleted [118].

Major protein depletion strategies include organic solvent precipitation with methanol, acetonitrile, isopropanol or acetone/trichloroacetic acid [119–121] and affinity dye-based depletion (Cibacron Blue). These methods substantially remove the most abundant protein in serum (albumin) but they act in a nonspecific manner and may co-deplete low-abundant glycoproteins that are bound to albumin [115]. They also suffer from a lack in reproducibility in the amount of proteins depleted [106,118]. Nonetheless, they have high loading capacity and low cost. Removal of immunoglobulins can be performed through affinity chromatography using protein A, protein G, protein L or the lectin concanavalin A. The binding is selective according to the immunoglobulin class [106]. Antibody-based methods are highly specific, especially when monoclonal antibodies are used, but are expensive and have low sample capacity [115,118]. More recently, combinatorial peptide ligand libraries have been successfully used to remove high-abundant proteins without losing the low-abundant components, allowing to equalize the amounts between high- and low-abundant proteins and glycoproteins. This reduces the dynamic range of concentrations in serum, facilitating the detection of low-abundant molecules [122,123].

Several immunoaffinity-based techniques are commercially available in the form of kits where the selective molecule is immobilized onto different supports and, through a chromatography-like procedure, incubation with the sample leads to a selective capture of the proteins/glycoproteins of interest. Examples include ProteomeLab IgY (Beckman Coulter), Seppro IgY and SuperMix (Sigma-Aldrich), and Multiple Affinity Removal System (MARS, Agilent Technologies). These commercial kits present the advantages of high reproducibility and partial automation, but a frequent limitation is the loading capacity (usually 1 ml of serum or plasma or less) that restrains the available processed sample to be analyzed or obligates to use several columns to process higher volumes of sample, significantly increasing the cost of the sample processing step. Their depleting performance has been compared [124].

To enrich the glycoproteins or glycans of interest, one of the most employed techniques is lectin affinity chromatography, where particular glycan structures are captured by the lectin with affinity for the referred structure [125–130]. The great variety of lectins enables to design procedures to enrich practically all possible glycan structures, which confers high flexibility to the methodology. Lectins can be purchased already bound to solid supports and lab-made systems can be implemented to perform lectin-affinity chromatography in a relatively automated way [131].

5.4. Biosensor selectivity

Biosensor selectivity is especially important when complex samples are to be analyzed, such as biological ones. Nonspecific interactions can occur, due to unspecific adsorption of matrix components (proteins, for example) on hydrophobic surfaces and components of the biosensor other than the lectin. To avoid these interactions, the biosensor may be incubated with polymers or surfactants like polyethylene glycol, polyvinyl alcohol, bovine serum albumin, gelatine, Tween 20 and SDS that form a layer covering the hydrophobic surface still accessible, impairing protein adsorption [75,78,132–136]. The use of mixed SAMs also enables the reduction of protein adsorption at electrode surfaces [137]. Ethanolamine is frequently used to block terminal active carboxylic sites in alkanethiols that were not occupied by the lectin [80,82,83].

In spite of all the blocking agents that can be used to optimize the lectin biosensor performance, we must keep in mind that analysis of serum samples always presents some difficulties because, even for a processed sample, the wide variety of existing glycoproteins together with the non-full specificity of each lectin impairs a 100% specific behaviour of the lectin biosensor.

6. Biosensor validation

Biosensor validation is a very long and complex process which aims to demonstrate that the biomarker test is safe and effective for the specific intended use or claim (such as diagnosis, prediction, prognosis or treatment monitoring) [138,139]. Through the validation process, both analytical and clinical performance must be extensively characterized and the clinical utility undoubtedly demonstrated, for the established target population. It has to be clearly established its clinical intended use, by assessing the validity, portability and reproducibility of the biomarker assay in diverse samples from various laboratories and clinics. Sensitivity and specificity of the biomarker assay must be determined with and without the presence of other diseases, to assess possible interferences [56]. Excellent and detailed descriptions of the validation process, its issues and challenges for official approval can be consulted in other papers [54,139–141].

Establishing the analytical and clinical validity of biomarker assays can take years and the majority of the discovered biomarkers fail in the validation tests. To illustrate the bottleneck that the validation process is, in 2011 there were 7720 publications on biomarkers usage, but only 407 of those were patented [142]. From those patented biomarkers, none had FDA approval [56]. In another study, from a list of 1261 proteins that have been cited in literature as being differentially expressed in human cancers, only 22% were reported to be present in the blood and could be detectable using a sensitive assay, and only 5% of those candidates were investigated as biomarkers, with around 3% being used in some clinical application [143].

The rate of successful clinical translation of biomarkers is estimated to be 0.1% [144]. These low numbers are a reflection of the lengthy process of assay development and validation, lack of reproducible data supporting clinical application and limited support by industry for these efforts, especially in the performance of prospective clinical trials [145,146].

7. Today's challenges for real implementation of lectin-based biosensors

Although the methodology for the construction of lectin biosensors has been well-established, some practical aspects still need to be improved, namely:

(a) Increase the robustness of the devices so that they can be prepared and stored to be used later, allowing mass production, transportation and storage. At the present moment, the proposed lectin-based biosensors reported in literature still suffer from some lack of robustness. Biosensors must be used in sample analysis in the same day or in the following days after their construction, and storage must be carried out in buffer solutions at 4 °C to preserve lectin activity. This disables long-distance transportation and mass production, impairing commercialization.

(b) Increase the reproducibility of measurements (this must be also considered for the fabrication of the employed supports, such as screen-printed electrodes). On one hand, screen-printed electrodes frequently used as platforms to construct lectin-biosensors show some irreproducibility in their electrochemical behaviour, even within the same fabrication lot, which will affect the final reproducibility of the lectin biosensor. On the other hand, if the devices are meant to be of single-use, the construction process must demonstrate that the same sample analyzed by different biosensors, produced in different days, originates the same result.

(c) Develop strategies for biosensor regeneration or decrease the construction costs, to allow massive implementation, including for groups and individuals with constrained resources. If the aim of lectin-based biosensors is to be used routinely in the clinic, costs in their massive production must be reduced so that they can be affordable to the health systems. Currently, the lectin biosensors proposed in literature are of single-use, which has the advantage of avoiding cross-contamination between samples but, on the other hand, increases the number of biosensors needed (one per measurement). In single-use mode, regeneration does not make sense. Nevertheless, if regeneration procedures are developed and demonstrate to be efficient in surface cleaning and keeping intact the lectin binding capacity, this would reduce enormously the costs associated with the production.

(d) Develop equipments that allow portability of the measurements (in a similar way to the glucose and cholesterol meters). Presently, the proposed lectin-based biosensors are small devices but, to perform measurements, they need to be connected to potentiostats or other instruments which are not portable equipments. If the aim is to use these biosensors in POC places (the doctor's office or the hospital ward), portable devices that include the biosensor and the measuring equipment are required. A possible solution could imitate the glucose meters and could also include fluidic-based platforms that manipulate very small body fluids with precision, especially those built from low-cost materials.

(e) Fulfil the sensitivity and specificity requirements. The lack of 100% specificity between a lectin and a glycan, in opposition to the unique and exclusive relation between an antigen and the corresponding antibody, is still problematic for practical applications and this limitation can impair the use of a lectin biosensor in sample analysis, where the matrices are extremely complex. Future directions may focus on identifying new and more specific carbohydrate biomarkers that appear only in target cancer cells or body fluids of cancer patients, or developing multi-lectin biosensors in which a glycoprofile and not a single glycobiomarker is monitored. This strategy could improve the biosensor performance, reducing false positives and negatives. Another approach could be the development of biosensors incorporating lectins and antibodies, which could enable the same outcome.

(f) Extensive biosensor validation, through the analysis of a higher number of samples, in retrospective and prospective assays, to assess the utility and evaluate the performance of the developed biosensors in real life [36]. A major validation process must be carried out for the devices that look promising at the laboratory/prototype level.

Only when these points are accomplished, we can consider the possibility of employing commercial lectin biosensors in real clinic scenarios, enabling a POC analysis, near the patient and near the doctor, helping in disease diagnostic and prognostic, and in therapy monitoring.

8. Future trends

Research in the biosensor field makes sense only if the intention is to apply the developed biosensors in the real world. So far, numerous papers have been published describing new biosensors for a great diversity of biomarkers and applications, but all the research has resulted in very few products. The little real progress noted by some editorial views has to do with the poor precision and accuracy, the insufficient detection limits, the problems with calibration in use and high cost per measurement. In addition, many developed biosensors are not needed, since other methodologies with more attractive features are available, such as chromatography, MS and immunoassays. The need should be the driving force to develop a new biosensor [147].

It is well-known that an ideal marker, with 100% sensitivity and specificity, does not exist. Intra- and inter-individual biological variation and analytical imprecision affect reference values for serum tumour markers. Furthermore, the use of cancer biomarkers in the diagnosis, disease progression assessment, prognosis and treatment monitoring must be performed criteriously by clinicians, avoiding the obsessed search for genetic susceptibility markers and the intensive use of early diagnostic methods in particular groups of population, which result to be inadequate for their age and non-effective in the cost-benefit relation.

As cancer is a very complex and heterogeneous group of diseases, with high inter- and intra-variability, biosensors for a profile of biomarkers (a cancer biomarker signature) have more potential to be useful in the clinic since, in that case, higher selectivity and sensitivity may be achieved, compared to single-biomarker biosensors. Several lectins can be used to construct those biosensors, allowing to obtain a glycoprofile [148].

Integration of genomic and proteomic research with glycomics may facilitate the distinction between different cancer subtypes and cancer stages. Gathering information from these areas will improve the search for better cancer biomarkers and to establish cancer signatures that include genes, proteins and glycans associated with cancer.

The availability of high quality specimens, adequately collected and preserved, along with the solving of technical shortcomings and lack of experience of the technicians, will also help in the glycobiomarkers discovery and validation phases. The inclusion of sample pre-treatment steps together with the biosensor device in integrated analytical platforms, for example, based in flow systems, will decrease errors in sample handling and increase robustness and reproducibility in the analysis [8].

Collaborations among researchers in diverse fields related to biosensor production are essential to produce accurate and robust devices and partnerships between researchers and industry will be necessary for mass production and commercialization [149].

Lectin-based biosensors, in particular, have their place as new devices for the selective detection and quantification of cancer cells or aberrant glycoproteins through the interaction established between cancer-specific glycans and the lectin. The general advantages of biosensors favour their implementation in real clinic environments. Nonetheless, the present lectin biosensors have shown to be successful only from a proof-of-concept perspective. The next step, which is the

thorough ample validation with different sample sets, is mandatory to finally have a clinical application.

Conflict of interest declaration

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References

[1] D.R. Thévenot, K. Toth, R.A. Durst, G.S. Wilson, Electrochemical biosensors: recommended definitions and classification, Biosens. Bioelectron. 16 (2001) 121–131.
[2] K. Cammann, Bio-sensors based on ion-selective electrodes, Fresenius Z. Anal. Chem. 287 (1977) 1–9.
[3] A.P.F. Turner, I. Karube, G.S. Wilson (Eds.), Biosensors: Fundamentals and Applications, Oxford University Press, Oxford, 1987.
[4] F.-G. Bănică, Chemical Sensors and Biosensors: Fundamentals and Applications, Wiley, United Kingdom, 2012.
[5] P. D'Orazio, Biosensors in clinical chemistry, Clin. Chim. Acta 334 (2003) 41–69.
[6] J. Lin, H. Ju, Electrochemical and chemiluminescent immunosensors for tumor markers, Biosens. Bioelectron. 20 (2005) 1461–1470.
[7] J. Wang, Electrochemical biosensors: towards point-of-care cancer diagnostics, Biosens. Bioelectron. 21 (2006) 1887–1892.
[8] S.A. Soper, K. Brown, A. Ellington, B. Frazier, G. Garcia-Manero, V. Gau, S.I. Gutman, D.F. Hayes, B. Korte, J.L. Landers, D. Larson, F. Ligler, A. Majumdar, M. Mascini, D. Nolte, Z. Rosenzweig, J. Wang, D. Wilson, Point-of-care biosensor systems for cancer diagnostics/prognostics, Biosens. Bioelectron. 21 (2006) 1932–1942.
[9] M.S. Belluzo, M.E. Ribone, C.M. Lagier, Assembling amperometric biosensors for clinical diagnostics, Sensors 8 (2008) 1366–1399.
[10] Y. Wang, H. Xu, J. Zhang, G. Li, Electrochemical sensors for clinical analysis, Sensors 8 (2008) 1043–2081.
[11] M. Mascini, S. Tombelli, Biosensors for biomarkers in medical diagnostics, Biomarkers 13 (2008) 637–657.
[12] M.R. Plata, A.M. Contento, A. Ríos, State-of-the-Art of (Bio)Chemical sensor developments in analytical Spanish groups, Sensors 10 (2010) 2511–2576.
[13] J. Li, S. Li, C.F. Yang, Electrochemical biosensors for cancer biomarker detection, Electroanalysis 24 (2012) 2213–2229.
[14] S. Patel, R. Nanda, S. Sahoo, E. Mohapatra, Biosensors in health care: the milestones achieved in their development towards lab-on-chip-analysis, Biochem. Res. Int. 2016 (2016) 1–12.
[15] P. Mehrotra, Biosensors and their applications – a review, J. Oral Biol. Craniofac. Res. 6 (2016) 153–159.
[16] Z. Altintas, W.M. Fakanya, I.E. Tothill, Cardiovascular disease detection using biosensing techniques, Talanta 128 (2014) 177–186.
[17] E.B. Bahadır, M.K. Sezgintürk, Electrochemical biosensors for hormone analyses, Biosens. Bioelectron. 68 (2015) 62–71.
[18] J. Masson, Surface plasmon resonance clinical biosensors for medical diagnostics, ACS Sens. 2 (2017) 16–30.
[19] G. Selvolini, G. Marrazza, MIP-based sensors: promising new tools for cancer biomarker determination, Sensors 17 (2017) 1–19.
[20] F. Zhang, S.S. Cho, S.H. Yang, S.S. Seo, G.S. Cha, H. Nam, Gold nanoparticle-based mediatorless biosensor prepared on microporous electrode, Electroanalysis 18 (2006) 217–222.
[21] F. Zhang, S.H. Yang, T.Y. Kang, G.S. Cha, H. Nam, M.E. Meyerhoff, A rapid competitive binding nonseparation electrochemical enzyme immunoassay (NEEIA) test strip for microcystin-LR (MCLR) determination, Biosens. Bioelectron. 22 (2007) 1419–1425.
[22] J.S. Daniels, N. Pourmand, Label-free impedance biosensors: opportunities and challenges, Electroanalysis 19 (2007) 1239–1257.
[23] D. Pihíková, P. Kasák, J. Tkac, Glycoprofiling of cancer biomarkers: label-free electrochemical lectin-based biosensors, Open Chem 13 (2015) 636–655.
[24] D. Janasek, J. Franzke, A. Manz, Scaling and the design of miniaturized chemical-analysis systems, Nature 442 (2006) 374–380.
[25] A. Rasooly, J. Jacobson, Development of biosensors for cancer clinical testing, Biosens. Bioelectron. 21 (2006) 1851–1858.
[26] A. Carlsson, C. Wingren, J. Ingvarsson, P. Ellmark, B. Baldertorp, M. Fernö, H. Olsson, C.A.K. Borrebaeck, Serum proteome profiling of metastatic breast cancer using recombinant antibody microarrays, Eur. J. Canc. 44 (2008) 472–480.
[27] J. Ingvarsson, C. Wingren, A. Carlsson, P. Ellmark, B. Wahren, G. Engström, U. Harmenberg, M. Krogh, C. Peterson, C.A.K. Borrebaeck, Detection of pancreatic cancer using antibody microarray-based serum protein profiling, Proteomics 8 (2008) 2211–2219.
[28] J.F. Rusling, C.V. Kumar, J.S. Gutkind, V. Patel, Measurement of biomarker proteins for point-of-care early detection and monitoring of cancer, Analyst 135 (2010) 2496–2511.
[29] J.D. Cohen, A.A. Javed, C. Thoburn, F. Wong, J. Tie, P. Gibbs, C.M. Schmidt, M.T. Yip-Schneider, P.J. Allen, M. Schattner, R.E. Brand, A.D. Singhi, G.M. Petersen, S. Hong, S.C. Kim, M. Falconi, C. Doglioni, M.J. Weiss, N. Ahuja, J. He, M.A. Makary, A. Maitra, S.M. Hanash, M. Dal Molin, Y. Wang, L. Li, J. Ptak, L. Dobbyn, J. Schaefer, N. Silliman, M. Popoli, M.G. Goggins, R.H. Hruban, C.L. Wolfgang, A.P. Klein, C. Tomasetti, N. Papadopoulos, K.W. Kinzler, B. Vogelstein, A.M. Lennon, Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 10202–10207.
[30] J.D. Cohen, L. Li, Y. Wang, C. Thoburn, B. Afsari, L. Danilova, C. Douville, A.A. Javed, F. Wong, A. Mattox, R.H. Hruban, C.L. Wolfgang, M.G. Goggins, M. Dal Molin, T. Wang, R. Roden, A.P. Klein, J. Ptak, L. Dobbyn, J. Schaefer, N. Silliman, M. Popoli, J.T. Vogelstein, J.D. Browne, R.E. Schoen, R.E. Brand, J. Tie, P. Gibbs, H. Wong, A.S. Mansfield, J. Jen, S.M. Hanash, M. Falconi, P.J. Allen, S. Zhou, C. Bettegowda, L. Diaz, C. Tomasetti, K.W. Kinzler, B. Vogelstein, A.M. Lennon, N. Papadopoulos, Detection and localization of surgically resectable cancers with a multi-analyte blood test, Science 359 (2018) 926–930.
[31] J.A. Ludwig, J.N. Weinstein, Biomarkers in cancer staging, prognosis and treatment selection, Nat. Cancer Rev. 5 (2005) 845–856.
[32] G. Danaei, S.V. Hoorn, A.D. Lopez, C.J.L. Murray, M. Ezzati, Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors, Lancet 366 (2005) 1784–1793.
[33] K. Smetana Jr., L. Lacina, P. Szabo, B. Dvořánková, P. Brož, A. Šedo, Ageing as an important risk factor for cancer, Anticancer Res. 36 (2016) 5009–5017.
[34] G. Rennert, Cancer prevention: from public health interventions to individual tailoring, Eur. J. Canc. Prev. 16 (2007) 165–166.
[35] M.J. Duffy, Tumor markers in clinical practice: a review focusing on common solid cancers, Med. Princ. Pract. 22 (2012) 4–11.
[36] M.J. Duffy, Use of biomarkers in screening for cancer, in: R. Scatena (Ed.), Advances in Cancer Biomarkers, Advances in Experimental Medicine and Biology, Springer, Dordrecht, 2015, pp. 27–39.
[37] S. Marella, Prognostic and predictive markers in early detection of different types of cancers for selected organ sites, J. Pharm. Biol. Sci. 8 (2013) 25–42.
[38] British Association of Surgical Oncology Guidelines, The management of metastatic bone disease in the United Kingdom. The breast specialty group of the British association of surgical oncology, Eur. J. Surg. Oncol. 25 (1999) 3–23.
[39] A. Kirwan, M. Utratna, M.E. O'Dwyer, L. Joshi, M. Kilcoyne, Glycosylation-based serum biomarkers for cancer diagnostics and prognostics, BioMed. Res. Int. 2015 (2015) 1–16.
[40] A. Szilvás, A. Blázovics, G. Székely, E. Dinya, J. Fehér, G. Mózsik, Comparative study between the free radicals and tumor markers in patients with gastrointestinal tumors, J. Physiol. 95 (2001) 247–252.
[41] T. Schöndorf, M. Hoopmann, M. Warm, R. Neumann, A. Thomas, U.J. Göhring, C. Eisberg, P. Mallmann, Serologic concentrations of HER-2/neu in breast cancer patients with visceral metastases receiving trastuzumab therapy predict the clinical course, Clin. Chem. 48 (2002) 1360–1362.
[42] S. Hakomori, Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines, Adv. Exp. Med. Biol. 491 (2001) 369–402.
[43] S. Hakomori, Glycosylation defining cancer malignancy: new wine in an old bottle, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 10231–10233.
[44] Y.J. Kim, A. Varki, Perspectives on the significance of altered glycosylation of glycoproteins in cancer, Glycoconj. J. 14 (1997) 569–576.
[45] S.A. Brooks, T.M. Carter, L. Royle, D.J. Harvey, S.A. Fry, C. Kinch, R.A. Dwek, P.M. Rudd, Altered glycosylation of proteins in cancer: what is the potential for new anti-tumour strategies, Anticancer Agents Med. Chem. 8 (2008) 2–21.
[46] S.S. Pinho, C.A. Reis, Glycosylation in cancer: mechanisms and clinical implications, Nat. Rev. Canc. 15 (2015) 540–555.
[47] S. Hakomori, Tumor malignancy defined by aberrant glycosylation and sphingo (glyco)lipid metabolism, Can. Res. 56 (1996) 5309–5318.
[48] H.J. An, T.R. Peavy, J.L. Hedrick, C.B. Lebrilla, Determination of N-Glycosylation sites and site heterogeneity in glycoproteins, Anal. Chem. 75 (2003) 5628–5637.
[49] C.B. Lebrilla, H.J. An, The prospects of glycan biomarkers for the diagnosis of diseases, Mol. Biosyst. 5 (2009) 17–20.
[50] P.M. Drake, W. Cho, B. Li, A. Prakobphol, E. Johansen, N.L. Anderson, F.E. Regnier, B.W. Gibson, S.J. Fisher, Sweetening the pot: adding glycosylation to the biomarker discovery equation, Clin. Chem. 56 (2010) 223–236.
[51] C. Ohyama, M. Hosono, K. Nitta, M. Oh-eda, K. Yoshikawa, T. Habuchi, Y. Arai, M. Fukuda, Carbohydrate structure and differential binding of prostate specific antigen to Maackia amurensis lectin between prostate cancer and benign prostate hypertrophy, Glycobiology 14 (2004) 671–679.
[52] A.R. Vashi, K.J. Wojno, W. Henricks, B.A. England, R.L. Vessella, P.H. Lange, G.L. Wright Jr., P.F. Schellhammer, R.A. Weigand, R.M. Olson, B.L. Dowell, K.K. Borden, J.E. Oesterling, Determination of the "reflex range" and appropriate cutpoints for percent free prostate-specific antigen in 413 men referred for prostatic evaluation using the AxSYM system, Urology 49 (1997) 19–27.
[53] S.A. Svarovsky, L. Joshi, Cancer glycan biomarkers and their detection – past, present and future, Anal. Methods 6 (2014) 3918–3936.
[54] A.K. Füzéry, J. Levin, M.M. Chan, D.W. Chan, Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges, Clin. Proteonomics 10 (2013) 1–14.
[55] H.A. Badr, D.M.M. AlSadek, A.A. Darwish, A.I. ElSayed, B.O. Bekmanov, E.M. Khussainova, X. Zhang, W.C.S. Cho, L.B. Djansugurova, C.-Z. Li, Lectin approaches for glycoproteomics in FDA-approved cancer biomarkers, Expert Rev. Proteomics 11 (2014) 227–236.

[56] S. Yotsukura, H. Mamitsuka, Evaluation of serum-based cancer biomarkers: a brief review from a clinical and computational viewpoint, Crit. Rev. Oncol. Hematol. 93 (2015) 103–115.
[57] U. Kuzmanov, H. Kosanam, E.P. Diamandis, The sweet and sour of serological glycoprotein tumor biomarker quantification, BMC Med. 11 (2013) 1–14.
[58] G. Sánchez-Pomales, R.A. Zangmeister, Recent advances in electrochemical glycobiosensing, Int. J. Electrochem. 2011 (2011) 1–11.
[59] A.M. Bielik, J. Zaia, Historical overview of glycoanalysis, Meth. Mol. Biol. 600 (2010) 9–30.
[60] A.F.S. Santos, M.D.C. Silva, T.H. Napoleão, P.M.G. Paiva, M.T.S. Correia, L.C.B.B. Coelho, Lectins: function, structure, biological properties and potential applications, Curr. Top. Pept. Protein Res. 15 (2014) 41–62.
[61] O.H. Hashim, J.J. Jayapalan, C.-S. Lee, Lectins: an effective tool for screening of potential cancer biomarkers, Peer J. 5 (2017) 1–30.
[62] L.C.B.B. Coelho, P.M.S. Silva, V.L.M. Lima, E.V. Pontual, P.M.G. Paiva, T.H. Napoleão, M.T.S. Correia, Lectins, interconnecting proteins with biotechnological/pharmacological and therapeutic applications, Evid. Based Complement. Alternative Media 2017 (2017) 1–22.
[63] R. Loris, T. Hamelryck, J. Bouckaert, L. Wyns, Legume lectin structure, Biochim. Biophys. Acta 1383 (1998) 9–36.
[64] M. Mammen, S. Choi, G.M. Whitesides, Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors, Angew. Chem. Int. Ed. 37 (1998) 2754–2794.
[65] J.C. Sacchettini, L.G. Baum, C.F. Brewer, Multivalent protein-carbohydrate interactions. A new paradigm for supermolecular assembly and signal transduction, Biochemistry 40 (2001) 3009–3015.
[66] C.F. Brewer, T.K. Dam, Binding and cross-linking properties of galectins, Biochim. Biophys. Acta 1572 (2002) 255–262.
[67] F.S. Coulibaly, B.-B.C. Youan, Current status of lectin-based cancer diagnosis and therapy, AIMS Mol. Sci. 4 (2017) 1–27.
[68] T. Kumada, S. Nakano, I. Takeda, S. Kiriyama, Y. Sone, K. Hayashi, H. Katoh, T. Endoh, T. Sassa, S. Satomura, Clinical utility of *Lens culinaris* agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: special reference to imaging diagnosis, J. Hepatol. 30 (1999) 125–130.
[69] A. Leerapun, S.V. Suravarapu, J.P. Vida, R.J. Clark, E.L. Sanders, T.A. Mettler, L.M. Stadheim, I. Aderca, C.D. Moser, D.M. Nagorney, N.F. LaRusso, P.C. de Groen, K.V. Menon, K.N. Lazaridis, G.J. Gores, M.R. Charlton, R.O. Roberts, T.M. Therneau, J.A. Katzmann, L.R. Roberts, The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population, Clin. Gastroenterol. Hepatol. 5 (2007) 394–402.
[70] B. Milutinovic, M. Jankovic, Analysis of the protein and glycan parts of CA125 antigen from human amniotic fluid, Arch. Biol. Sci. 59 (2007) 97–103.
[71] K. Chen, A. Gentry-Maharaj, M. Burnell, C. Steentoft, L. Marcos-Silva, U. Mandel, I. Jacobs, A. Dawnay, U. Menon, O. Blixt, Microarray glycoprofiling of CA125 improves differential diagnosis of ovarian cancer, J. Proteome Res. 12 (2013) 1408–1418.
[72] H. Ideo, Y. Hinoda, K. Sakai, I. Hoshi, S. Yamamoto, M. Oka, K. Maeda, N. Maeda, S. Hazama, J. Amano, K. Yamashita, Expression of mucin 1 possessing a 3'-sulfated core 1 in recurrent and metastatic breast cancer, Int. J. Canc. 137 (2015) 1652–1660.
[73] A. Nagata, M. Miura, T. Komoda, Sandwich assay for carcinoembryonic antigen with immobilized lectins and a monoclonal antibody, Tumour Biol 12 (1991) 35–44.
[74] F.-Y. Zeng, A. Benguría, S. Kafert, S. André, H.-J. Gabius, A. Villalobo, Differential response of the epidermal growth factor receptor tyrosine kinase activity to several plant and mammalian lectins, Mol. Cell. Biochem. 142 (1995) 117–124.
[75] D. Pihikova, Z. Pakanova, M. Nemcovic, P. Barath, S. Belicky, T. Bertok, P. Kasak, J. Mucha, J. Tkac, Sweet characterisation of prostate specific antigen using electrochemical lectin-based immunosensor assay and MALDI TOF/TOF analysis: focus on sialic acid, Proteomics 16 (2016) 3085–3095.
[76] T. Kanai, M. Amakawa, R. Kato, K. Shimizu, K. Nakamura, K. Ito, Y. Hama, M. Fujimori, J. Amano, Evaluation of a new method for the diagnosis of alterations of Lens culinaris agglutinin binding of thyroglobulin molecules in thyroid carcinoma, Clin. Chem. Lab. Med. 47 (2009) 1285–1290.
[77] A. Takeya, O. Hosomi, H. Nishijima, Y. Ohe, K. Sugahara, M. Sagi, K. Yamazaki, H. Hayakawa, H. Takeshita, C. Sasaki, T. Kogure, T. Mukai, Presence of beta-linked GalNAc residues on N-glycans of human thyroglobulin, Life Sci. 80 (2007) 538–545.
[78] T. Bertók, J. Katrlík, P. Gemeiner, J. Tkac, Electrochemical lectin based biosensors as a label-free tool in glycomics, Microchim. Acta 180 (2013) 1–13.
[79] Z. Dai, A.-N. Kawde, Y. Xiang, J.T. LaBelle, J. Gerlach, V.P. Bhavanandan, L. Joshi, J. Wang, Nanoparticle-based sensing of glycan-lectin interactions, J. Am. Chem. Soc. 128 (2006) 10018–10019.
[80] J.T. La Belle, J.Q. Gerlach, S. Svarovsky, L. Joshi, Label-free impedimetric detection of glycan-lectin interactions, Anal. Chem. 79 (2007) 6959–6964.
[81] T. Bertók, A. Sediva, J. Katrlik, P. Gemeiner, M. Miluka, M. Nosko, J. Tkac, Label-free detection of glycoproteins by the lectin biosensor down to attomolar level using gold nanoparticles, Talanta 108 (2013) 11–18.
[82] M.L.S. Silva, E. Gutiérrez, J.A. Rodríguez, C. Gomes, L. David, Construction and validation of a Sambucus nigra biosensor for cancer-associated STn antigen, Biosens. Bioelectron. 57 (2014) 254–261.
[83] M.L.S. Silva, M.G.H. Rangel, A Vicia villosa agglutinin biosensor for cancer-associated Tn antigen, Sens. Actuators, B 252 (2017) 777–784.
[84] K.J. Foley, E.S. Forzani, J. Joshi, N. Tao, Detection of lectin-glycan interaction using high resolution surface plasmon resonance, Analyst 133 (2008) 744–746.
[85] V.J. Nagaraj, S. Aithal, S. Eaton, M. Bothara, P. Wiktor, S. Prasad, NanoMonitor: a miniature electronic biosensor for glycan biomarker detection, Nanomedicine 5 (2010) 369–378.
[86] H. Yang, Z. Li, X. Wei, R. Huang, H. Qi, Q. Gao, C. Li, C. Zhang, Detection and discrimination of alpha-fetoprotein with a label-free electrochemical impedance spectroscopy biosensor array based on lectin functionalized carbon nanotubes, Talanta 111 (2013) 62–68.
[87] C. Yang, C. Xu, X. Wang, X. Hu, Quantum-dot-based biosensor for simultaneous detection of biomarker and therapeutic drug: first steps toward an assay for quantitative pharmacology, Analyst 137 (2012) 1205–1209.
[88] L. Zhao, C. Li, H. Qi, Q. Gao, C. Zhang, Electrochemical lectin-based biosensor array for detection and discrimination of carcinoembryonic antigen using dual amplification of gold nanoparticles and horseradish peroxidase, Sens. Actuators, B 235 (2016) 575–582.
[89] L.S. Kelly, S. Birken, D. Puett, Determination of hyperglycosylated human chorionic gonadotropin produced by malignant gestational trophoblastic neoplasias and male germ cell tumors using a lectin-based immunoassay and surface plasmon resonance, Mol. Cell. Endocrinol. 260–262 (2007) 33–39.
[90] Y.M. Park, S.J. Kim, K. Kim, Y.D. Han, S.S. Yang, H.C. Yoon, Lectin-based optical sensing for quantitative analysis of cancer antigen CA15-3 as a breast cancer marker, Sens. Actuators, B 186 (2013) 571–579.
[91] S. Cunningham, J.Q. Gerlach, M. Kane, L. Joshi, Glyco-biosensors: recent advances and applications for the detection of free and bound carbohydrates, Analyst 135 (2010) 2471–2480.
[92] W. Putzbach, N.J. Ronkainen, Immobilization techniques in the fabrication of nanomaterial-based electrochemical biosensors: a review, Sensors 13 (2013) 4811–4840.
[93] W.H. Scouten, J.H.T. Luong, R.S. Brown, Enzyme or protein immobilization techniques for applications in biosensor design, Trends Biotechnol. 13 (1995) 178–185.
[94] J.P. Hart, S.A. Wring, Recent developments in the design and application of screen-printed electrochemical sensors for biomedical, environmental, and industrial analyses, Trends Anal. Chem. 16 (1997) 89–103.
[95] I. Palchetti, A. Cagnini, M.D. Carlo, C. Coppi, M. Mascini, A.P.F. Turner, Determination of anticholinesterase pesticides in real samples using a disposable biosensor, Anal. Chim. Acta 337 (1997) 315–321.
[96] M.P. O'Halloran, M. Pravda, G.G. Guilbault, Prussian Blue bulk modified screen-printed electrodes for H_2O_2 detection and for biosensors, Talanta 55 (2001) 605–611.
[97] N. Thiyagarajan, J.-L. Chang, K. Senthilkumar, J.-M. Zen, Disposable electrochemical sensors: a mini-review, Electrochem. Commun. 38 (2014) 86–90.
[98] J.C. Love, L.A. Estroff, J.K. Kriebel, R.G. Nuzzo, G.M. Whitesides, Self-assembled monolayers of thiolates on metals as a form of nanotechnology, Chem. Rev. 105 (2005) 1103–1170.
[99] P. Skládal, Advances in electrochemical immunosensors, Electroanalysis 9 (1997) 737–745.
[100] K.T. Pilobello, L. Krishnamoorthy, D. Slawek, L.K. Mahal, Development of a lectin microarray for the rapid analysis of protein glycopatterns, Chembiochem 6 (2005) 985–989.
[101] K.S. Nascimento, A.I. Cunha, K.S. Nascimento, B.S. Cavada, A.M. Azevedo, M.R. Aires-Barros, An overview of lectins purification strategies, J. Mol. Recogn. 25 (2012) 527–541.
[102] H.J. Gabius, Ca2+: mastermind and active player for lectin activity (including a gallery of lectin folds), in: H.J. Gabius (Ed.), The Sugar Code. Fundamentals of Glycosciences, Wiley-VCH, Weinheim, 2009, pp. 269–278.
[103] D. Caballero, E. Martinez, J. Bausells, A. Errachid, J. Samitier, Impedimetric immunosensor for human serum albumin detection on a direct aldehyde-functionalized silicon nitride surface, Anal. Chim. Acta 720 (2012) 43–48.
[104] J.A. Ferreira, A.L. Daniel-da-Silva, R.M.P. Alves, D. Duarte, I. Vieira, L.L. Santos, R. Vitorino, F. Amado, Synthesis and optimization of lectin functionalized nanoprobes for the selective recovery of glycoproteins from human body fluids, Anal. Chem. 83 (2011) 7035–7043.
[105] N.L. Anderson, N.G. Anderson, The human plasma proteome: history, character, and diagnostic prospects, Mol. Cell. Proteomics 1 (2002) 845–867.
[106] J.M. Jacobs, J.N. Adkins, W.-J. Qian, T. Liu, Y. Shen, D.G. Camp II, R.D. Smith, Utilizing human blood plasma for proteomic biomarker discovery, J. Proteome Res. 4 (2005) 1073–1085.
[107] R.L. Lundblad, Considerations for the use of blood plasma and serum for proteomic analysis, Internet J. Genom. Proteonomics 1 (2005) 1–8.
[108] W.P. Faulk, D.S. Torny, J.A. McIntyre, Effects of serum versus plasma on agglutination of antibody-coated indicator cells by human rheumatoid factor, Clin. Immunol. Immunopathol. 46 (1988) 169–176.
[109] G. Lum, S.R. Gambino, A comparison of serum *versus* heparinized plasma for routine clinical tests, Am. J. Clin. Pathol. 61 (1974) 108–113.
[110] M. West-Nielsen, E.V. Høgdall, E. Marchiori, C.K. Høgdall, C. Schou, N.H.H. Heegaard, Sample handling for mass spectrometric proteomic investigations of human sera, Anal. Chem. 77 (2005) 5114–5123.
[111] J. Villanueva, J. Philip, C.A. Chaparro, Y. Li, R. Toledo-Crow, L. DeNoyer, M. Fleisher, R.J. Robbins, P. Tempst, Correcting common errors in identifying cancer-specific serum peptide signatures, J. Proteome Res. 4 (2005) 1060–1072.
[112] C. Ricós, N. Iglesias, J.-V. García-Lario, M. Simón, F. Cava, A. Hernández, C. Perich, J. Minchinela, V. Alvarez, M.-V. Doménech, C.-V. Jiménez, C. Biosca, R. Tena, Within-subject biological variation in disease: collated data and clinical consequences, Ann. Clin. Biochem. 44 (2007) 343–352.
[113] J.N. Adkins, S.M. Varnum, K.J. Auberry, R.J. Moore, N.H. Angell, R.D. Smith, D.L. Springer, J.G. Pounds, Toward a human blood serum proteome: analysis by

multidimensional separation coupled with mass spectrometry, Mol. Cell. Proteomics 1 (2002) 947–955.
[114] C.-J. Tu, J. Dai, S.-J. Li, Q.-H. Sheng, W.-J. Deng, Q.-C. Xia, R. Zeng, High-sensitivity analysis of human plasma proteome by immobilized isoelectric focusing fractionation coupled to mass spectrometry identification, J. Proteome Res. 4 (2005) 1265–1273.
[115] M.-R. Kim, C.-W. Kim, Human blood plasma preparation for two-dimensional gel electrophoresis, J. Chromatogr. B 849 (2007) 203–210.
[116] D. Lin, W.E. Alborn, R.J.C. Slebos, D.C. Liebler, Comparison of protein immunoprecipitation-multiple reaction monitoring with ELISA for assay of biomarker candidates in plasma, J. Proteome Res. 12 (2013) 5996–6003.
[117] E. Bellei, S. Bergamini, E. Monari, L.I. Fantoni, A. Cuoghi, T. Ozben, A. Tomasi, High-abundance proteins depletion for serum proteomic analysis: concomitant removal of non-targeted proteins, Amino Acids 40 (2011) 145–156.
[118] L.A. Echan, H.-Y. Tang, N. Ali-Khan, K. Lee, D.W. Speicher, Depletion of multiple high-abundance proteins improves protein profiling capacities of human serum and plasma, Proteomics 5 (2005) 3292–3303.
[119] K. Merrell, K. Southwick, S.W. Graves, M.S. Esplin, N.E. Lewis, C.D. Thulin, Analysis of low-abundance, low-molecular-weight serum proteins using mass spectrometry, J. Biomol. Tech. 15 (2004) 238–248.
[120] Y.-Y. Chen, S.-Y. Lin, Y.-Y. Yeh, H.-H. Hsiao, C.-Y. Wu, S.-T. Chen, A.H.-J. Wang, A modified protein precipitation procedure for efficient removal of albumin from serum, Electrophoresis 26 (2005) 2117–2127.
[121] G. Liu, Y. Zhao, A. Angeles, L.L. Hamuro, M.E. Arnold, J.X. Shen, A novel and cost effective method of removing excess albumin from plasma/serum samples and its impacts on LC-MS/MS bioanalysis of therapeutic proteins, Anal. Chem. 86 (2014) 8336–8343.
[122] V. Thulasiraman, S. Lin, L. Gheorghiu, J. Lathrop, L. Lomas, D. Hammond, E. Boschetti, Reduction of the concentration difference of proteins in biological liquids using a library of combinatorial ligands, Electrophoresis 26 (2005) 3561–3571.
[123] P.G. Righetti, E. Boschetti, L. Lomas, A. Citterio, Protein Equalizer™ Technology*: the quest for a “democratic proteome”, Proteomics 6 (2006) 3980–3992.
[124] S. Roche, L. Tiers, M. Provansal, M. Seveno, M.-T. Piva, P. Jouin, S. Lehmann, Depletion of one, six, twelve or twenty major blood proteins before proteomic analysis: the more the better? J. Proteomics 72 (2009) 945–951.
[125] L. Xiong, F.E. Regnier, Use of a lectin affinity selector in the search for unusual glycosylation on proteomics, J. Chromatogr. B 782 (2002) 405–418.
[126] Z. Yang, W.S. Hancock, Approach to the comprehensive analysis of glycoproteins isolated from human serum using a multi-lectin affinity column, J. Chromatogr. A 1053 (2004) 79–88.
[127] Z. Yang, W.S. Hancock, Monitoring glycosylation pattern changes of glycoproteins using multi-lectin affinity chromatography, J. Chromatogr. A 1070 (2005) 57–64.
[128] Z. Yang, W.S. Hancock, T.R. Chew, L. Bonilla, A study of glycoproteins in human serum and plasma reference standards (HUPO) using multilectin affinity chromatography coupled with RPLC-MS/MS, Proteomics 5 (2005) 3353–3366.
[129] M. Madera, Y. Mechref, I. Klouckova, M.V. Novotny, High-sensitivity profiling of glycoproteins from human blood serum through multiple-lectin affinity chromatography and liquid chromatography/tandem mass spectrometry, J. Chromatogr. B 845 (2007) 121–137.
[130] K. Jung, W. Cho, F.E. Regnier, Glycoproteomics of plasma based on narrow selectivity lectin affinity chromatography, J. Proteome Res. 8 (2009) 643–650.
[131] M.L.S. Silva, C. Gomes, M.B.Q. Garcia, Flow lectin affinity chromatography – a model with *Sambucus nigra* agglutinin, J. Glycobiol. 5 (2016) 121–128.
[132] G.B. Sigal, M. Mrksich, G.M. Whitesides, Using surface plasmon resonance spectroscopy to measure the association of detergents with self-assembled monolayers of hexadecanethiolate on gold, Langmuir 13 (1997) 2749–2755.
[133] Z. Wang, A.S. Viana, G. Jin, L.M. Abrantes, Immunosensor interface based on physical and chemical immunoglobulin G adsorption onto mixed self-assembled monolayers, Bioelectrochemistry 69 (2006) 180–186.
[134] A.A.P. Ferreira, M.J.M. Alves, S. Barrozo, H. Yamanaka, A.V. Benedetti, Optimization of incubation time of protein Tc85 in the construction of biosensor: is the EIS a good tool? J. Electroanal. Chem. 643 (2010) 1–8.
[135] B. Liu, P.J. Huang, X. Zhang, F. Wang, R. Pautler, A.C. Ip, J. Liu, Parts-per-million of polyethylene glycol as a non-interfering blocking agent for homogeneous biosensor development, Anal. Chem. 85 (2013) 10045–10050.
[136] X. Luo, Q. Xu, T. James, J.J. Davis, Redox and label-free array detection of protein markers in human serum, Anal. Chem. 86 (2014) 5553–5558.
[137] F. Frederix, K. Bonroy, W. Laureyn, G. Reekmans, A. Campitelli, W. Dehaen, G. Maes, Enhanced performance of an affinity biosensor interface based on mixed self-assembled monolayers of thiols on gold, Langmuir 19 (2003) 4351–4357.
[138] S. Gutman, L.G. Kessler, The US Food and Drug Administration perspective on cancer biomarker development, Nat. Rev. Canc. 6 (2006) 565–571.
[139] N. Goossens, S. Nakagawa, X. Sun, Y. Hoshida, Cancer biomarker discovery and validation, Transl. Cancer Res. 4 (2015) 256–269.
[140] R.A. Beckman, C. Chen, Efficient, adaptive clinical validation of predictive biomarkers in cancer therapeutic development, in: R. Scatena (Ed.), Advances in Cancer Biomarkers. Advances in Experimental Medicine and Biology, Springer, Dordrecht, 2015, pp. 81–90.
[141] J.M. Rhea, R.J. Molinaro, Cancer Biomarkers: surviving the journey from bench to bedside, Med. Lab. Obs. 43 (2011) 10–12.
[142] E. Drucker, K. Krapfenbauer, Pitfalls and limitations in translation from biomarker discovery to clinical utility in predictive and personalized medicine, EPMA J. 13 (2013) 1–10.
[143] M. Polansky, N.L. Anderson, A list of candidate cancer biomarkers for targeted proteomics, Biomark. Insights 1 (2006) 1–48.
[144] G. Poste, Bring on the biomarkers, Nature 469 (2011) 156–157.
[145] R.C. Bast Jr., H. Lilja, N. Urban, D.L. Rimm, H. Fritsche, J. Gray, R. Veltri, G. Klee, A. Allen, N. Kim, S. Gutman, M.A. Rubin, A. Hruszkewycz, Translational crossroads for biomarkers, Clin. Canc. Res. 11 (2005) 6103–6108.
[146] M. Vidal, D.W. Chan, M. Gerstein, M. Mann, G.S. Omenn, D. Tagle, S. Sechi, The human proteome – a scientific opportunity for transforming diagnostics, therapeutics, and healthcare, Clin. Proteonomics 9 (2012) 1–11.
[147] P.T. Kissinger, Biosensors – a perspective, Biosens. Biolelectron. 20 (2005) 2512–2516.
[148] H. Chen, C. Jiang, C. Yu, S. Zhang, B. Liu, J. Kong, Protein chips and nanomaterials for application in tumor marker immunoassays, Biosens. Bioelectron. 24 (2009) 3399–3411.
[149] C.L. Sawyers, The cancer biomarker problem, Nature 452 (2008) 548–552.