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Nanoscale controlled architecture for development of ultrasensitive lectin biosensors applicable in glycomics

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Abstract

In this Minireview the most advanced patterning protocols and transducing schemes for development of ultrasensitive label-free and label-based lectin biosensors for glycoprofiling of disease markers and some cancerous cells are described. Performance of such lectin biosensors with interfacial properties tuned at a nanoscale are critically compared to the most sensitive immunoassay format of analysis and challenges ahead in the field are discussed. Moreover, key elements for future advances of such devices on the way to enhance robustness and practical applicability of lectin biosensors are revealed.

Glycomics

In recent years there is an ever-growing interest to switch from studying nucleic acids and proteins (genomics and proteomics) to more complex glycan structures (glycomics).^{1, 2} Glycans, often described as a third alphabet in molecular biology,³ are linked to glycolipids and glycoproteins in a linear or branched way and modulate their functions, stability and sorting inside a cell.⁴ Since 70% of all proteins are glycosylated⁵, structure and function of glycoproteins are intensively studied (glycoproteomics).⁶ Glycosylation is the most common co- and posttranslational modification⁷ triggered by the action of glycosyl transferases and glycosidases, representing about 2% of all translated genes.⁸ Moreover, a non-enzymatic modification of proteins is possible (glycation), as well. Glycans can be attached to proteins *via* –NH₂ (N-glycans) or –OH (O-glycans) groups.⁹ Glycans mediate biomolecular interactions, cell-cell and cell-matrix adhesion, viral infections and their modifications are often a consequence of a progression of a disease and glycans play a role in the development and functioning of a whole organism.^{10, 11} Moreover, advances in glycomics are applied for production of more efficient therapeutics with a controlled glycan composition by glycoengineering.¹²

Glycans can encode more complex information comparing to nucleic acids or proteins. The theoretical number of all possible glycan hexamers is 1.4×10^{15} , much larger compared to 6.4×10^6 for proteins and 4,096 for DNA, respectively, but it is estimated that a number of unique glycan sequences in human is in excess of 5,000.¹³ The study of the glycans often

requires the release of the glycan structure from the parental molecules using various chemical or enzymatic procedures.^{14, 15} Routinely used methods for the released glycan analysis are mass-spectrometry, capillary electrophoresis and liquid chromatography.^{16–18} A complementary approach relies on the application of lectins (natural glycan decipherers), which are able to specifically bind to various glycan sequences (Tab. 1) and in some cases even able to distinguish the bond between the two units (e.g. α or β , 2-3 or 2-6, etc. see Fig. 1).¹⁹ In comparison to conventional antibody or nucleic acid based recognition systems, requiring some knowledge about the analyte, lectin-based assay can be applied even, when the targets are not known and have been applied in finding new potential biomarkers of several diseases.^{19, 20} Smaller molecular size of lectins in comparison to antibodies allow the immobilisation of lectins at a higher density for a higher sensitivity/selectivity of assays compared to immunoassays.²⁰

These days, a routinely used and robust method for a direct glycoprofiling of intact proteins or cells with an extremely high throughput of analysis is a lectin microarray.^{2, 21} Although such detection platform helped to understand the role of glycans in physiological/pathological processes and revealed new prospective cancer biomarkers,^{22, 23} the technique offers a narrow concentration working range with a detection limit being in sub nM range.²⁴

In few recent years various sophisticated strategies in combination with lectins were launched to increase sensitivity and selectivity of assays or allowing to work even in a label-free format of analysis. Early developments in the field of lectin biosensors were shortly described in two excellent reviews published in 2010.^{25, 26} Later, progress achieved in the field of electrochemical glycan biosensing with few reports published in 2011 was described.²⁷ Two detailed reviews summarising development until the end of 2011 in the glycan-lectin biosensing⁷ and in application of nanoengineered glycan sensors²⁸ were published, as well. Recent advances in biorecognition, coming from Strano's group and only marginally covering lectin biosensors were published recently.²⁹ An excellent review dealing with potential of glycan biomarkers in cancer diagnosis was published by Joshi's group recently.³⁰

In this article attention will be paid to the most progressive discoveries in recent 3 years with a focus on advanced surface patterning protocols; promising label-free and label-based detection platforms, which can be further multiplexed; highly sensitive assay methods based on various labels; possible routes for addressed immobilisation of lectins within arrays; anticipated progress in engineering of lectins with value added functional properties and application of lectin biosensors as a possible diagnostic tool.

Lectin biosensors

Biosensors are devices integrating biorecognition molecules directly with a transducer, which converts a biorecognition event into a measurable signal.³¹ Thus, in this article lectin microarrays/biochips are not covered since in that case lectins are immobilised on glass slides, which are not a part of a measurement unit.³² Moreover, application of lectins in a homogeneous solution is not covered, as well. Lectin biosensors described here will be divided into two parts – working in a label-free mode or methods requiring any kind of label

– a redox or a fluorescent tag; and a nanoparticle (Fig. 2). When discussing performance of the biosensors, it is good to keep in mind that clinically relevant concentration of a prostate specific antigen, a biomarker for prostate cancer is 140 pM in the blood³³ and that lectin biosensors should have a limit of detection well below this level for an efficient work without any enrichment step.

Label-free lectin biosensors

There are many different bioassay methods available with some of them being destructive and requiring labelling of the recognition element or target molecules. This additional step would make the experiment relatively complex and could negatively affect the biorecognition event.⁷ Therefore, label-free concepts of analysis have attracted extensive interest for fabrication of biosensors. The most prospective label-free lectins biosensors relying on mechanical, electrochemical or optical signal readout are discussed here.

Electrochemical impedance spectroscopy (EIS) is one of quite a few electrochemical methods of detection³⁴, offering simplicity of operation and miniaturisation, low cost, low power consumption and a quick response. During EIS assays, a small sinusoidal potential perturbation of a thin biorecognition layer immobilised on an electrode surface is performed and data evaluation can provide information about interfacial properties of a receptive layer (i.e. a charge transfer resistance R_{CT} and a capacitance), which can be directly used to quantify analyte of interest.³

Joshi's group described first application of EIS-based lectin biosensors to detect glycoproteins with printed circuit board electrodes.³⁵ Analysis was quick and sensitive offering a limit of detection down to 15 fM and the method was recommended for point-of-care diagnosis of cancer at an early stage.³⁵ A follow up study utilised lectins immobilised on an array of gold electrodes within nanowells of a high density.³⁶ Moreover, the EIS-based lectin biosensor was more sensitive and quicker compared to traditionally used enzyme-linked lectin assays in analysis of glycoproteins.³⁶

In our group we focused on the development of ultrasensitive EIS-based lectin biosensors trying to outperform initial biosensor devices in term of sensitivity, offering a detection limit down to single-molecule level, what has been achieved by a controlled architecture of a receptive layer at a nanoscale.^{24, 37, 38} In our first study a 2-D lectin biosensor was constructed by a covalent immobilisation of a sialic acid recognising lectin (*Sambucus nigra* agglutinin) on a mixed self-assembled monolayer on gold. The biosensor was able to detect two sialic acid containing glycoproteins with a difference in the sensitivity of their detection proportional to the amount of sialic acid present within a glycan moiety on the protein surface. The device offered a working concentration range spanning 7 orders of magnitude with a detection limit for the glycoprotein down to 300 aM, which was the lowest glycoprotein concentration detected at the time of publication.³⁸ In our second study, the incorporation of gold nanoparticles for lectin immobilisation was behind preparation of a 3-D lectin biosensor offering even lower and an unprecedented detection limit of 0.5 aM with quite a wide dynamic concentration range covered.³⁷ In our last study lectin biosensors were constructed with three different lectins for analysis of glycan changes on immunoglobulins with progression of a rheumatoid arthritis in humans. The biosensor with

improved antifouling properties, based on a betaine functionality, offered a detection limit in a fM range and worked properly even in 1,000-fold diluted human plasma. The biosensor performance was directly compared to the state-of-the-art glycoprofiling tool based on fluorescent lectin microarrays with a detection limit in the nM level. A sandwich configuration applied in a biosensor assay protocol allowed detecting aM level of glycoproteins.²⁴

Carbon nanotubes were applied for a construction of ultrasensitive lectin biosensors for analysis of α -fetoprotein, a cancer biomarker, down to a concentration of 1 fM.³⁹ Moreover, other 4 lectins were subsequently integrated into a biosensor device for analysis of serum samples from healthy individuals and people having cancer, showing differences in a glycoprofile.³⁹ Oliveira's group focused on a successful discrimination between healthy human samples and samples from patients infected by a mosquito-borne Dengue virus (breakbone fever) with a high mortality rate using EIS-based lectin biosensors.³¹ Some biosensors were constructed with the aid of different nanoparticles and could detect pM level of an analyte.³¹

Besides analysis of glycoproteins, EIS lectin-based biosensors allow to detect glycans directly on the surface of intact cells. Only 5,000 human leukemic cells of a cell line K562 was needed for glycoprofiling by a microfluidic device with an array of four ITO electrodes modified by gold nanoparticles and three different lectins.⁴⁰ In addition to EIS detection, binding events on transparent ITO electrodes, binding events can be monitored by an optical microscope. Both methods of analysis were applied to determine composition of cell surface glycans in response to one drug and the results showed a general agreement between these two methods.⁴⁰ A human liver cancer cell line Bel-7404 was detected down to a concentration of 234 cells/mL by a label-free EIS sensing with Con A immobilised directly on a gold electrode, while a normal liver cell line exhibited a low response due to a low expression of a membrane-bound glycoprotein gp43.⁴¹ Moreover, the lectin biosensor offered a robust analysis with a recovery index of 97-100%.⁴¹

In all EIS-based lectin biosensors discussed above a redox probe was needed to get an electrochemical response. There is, however, one study, in which EIS response was obtained in a non-Faradaic mode of operation, but it was important to optimise the ionic strength of the electrolyte employed for measurement.⁴² This is why for analysis of ovarian cancer cells SKOV3, a dilute phosphate buffer was applied with a detection limit of only 4 captured cells.⁴²

EIS-based lectin biosensors proved to be as sensitive as the most sensitive EIS-based immunoassays, able to detect aM level of glycoproteins.⁴³ In order to compete with lectin microarray technology, EIS-based methods have to be developed to have high throughput of analysis for a multiplexed glycoprofiling. The other issue to be addressed in lectin biosensors is to apply EIS for a real time analysis, which is possible by working at a single frequency.⁴⁴

Field-effect transistor (FET) sensing is an emerging class of a label-free sensing format, applicable to a range of biological targets.⁴⁵ A FET biosensor device consists of a

semiconductor channel connecting two metal electrodes (source and drain) with a biorecognition element being immobilised on a semiconductor. Once a biorecognition event takes place, a change in conductivity of the semiconductor channel is used for the generation of the signal. There are other format of analysis involves a third gate electrode.²⁸ The only FET device with lectin immobilised on a surface of a single-walled carbon nanotubes (SWCNTs) was described recently,⁴⁶ but the device was not applied in analysis of glycoproteins. It is surprising, why such a detection platform has not been applied in the analysis of glycoproteins, since devices with antibodies immobilised can detect proteins down to aM level⁴⁷ or single virus particles from 80 aM solutions (i.e. 50 viruses/ μ L) in a multiplexed format of analysis.⁴⁸

Recent studies suggest that a SWCNT-based FET device compared to the graphene-based one had distinct advantages⁴⁹ and that silicon nanowires are better suited for a construction of FET devices compared to SWCNTs, offering a highly sensitive detection with a mass production of such nanostructures, benefiting from a well-developed semiconductor fabrication.⁵⁰

A capacitance assay protocol sampling the current response triggered by a small potentiostatic step at a frequency of 50 kHz launched by Berggren can be applied in a label-free electrochemical detection of a biorecognition event.⁵¹ This technique was used for analysis of a state of IgG, produced by a recombinant technology.⁵² It was proved that the method was able to detect aggregated form of IgG as low as 0.01% of a total IgG, what can be applied in a quality control of a recombinant protein production.⁵²

Surface plasmon resonance (SPR) is one of the most popular optical tools for monitoring of biological interactions. It is based on detection of a change in a refractive index of the medium in a close vicinity of a thin metal, mostly gold, surface exposed to light under a particular angle to achieve a total internal reflection.⁵³ Changes in a refractive index due to analyte binding to the immobilised ligand influence the resonant angle and this shift is used to generate a real-time signal. SPR allows not only the real-time analysis of interactions between biomolecules immobilised on the metal surface and unlabelled solution phase molecules, but it also provides additional information about affinity and kinetic parameters.⁵⁴

Danielsson and Safina applied SPR method to study an interaction of 6 glycoproteins with 6 lectins, but the assay was not particularly sensitive since the limit of detection is in the nM range. After finding proper regeneration conditions, the biosensor surface could be effectively reused and the method was applied to get kinetic and affinity constants of biorecognition events.⁵⁵ When SPR was applied in analysis of pathogenic *Escherichia coli* O157:H7, the method offered a detection limit of 3,000 cfu/mL (cfu=colony forming unit).⁵⁶

Results published so far suggest, that SPR-based lectin biosensors could not compete with lectin microarrays in terms of frequency and sensitivity of detection. In order to outperform lectin microarrays, SPR lectin biosensors have to be integrated into an array format of analysis (SPR imaging)⁵⁷ and to implement some amplification strategies.⁵⁸ On the other

side, SPR technology can be a key tool to characterise development of bioreceptive layers for construction of novel and highly sensitive biosensors.⁵⁹ Even though a localised surface plasmon resonance using metallic nanoparticles/nanoislands is quite a new method to study a biorecognition in a real time,⁶⁰ so far it can be applied for evaluation of strength of interaction rather than for a competitive glycoprotein detection.⁶¹

Quartz crystal microbalance (QCM) as another label-free method of detection, rather effective in getting kinetic/affinity constants of an interaction.⁶² Since sensitivity of detection is lower than in case of SPR and to achieve low detection limits for QCM-based biosensors an amplification strategy⁶³ or a displacement mode of operation⁶⁴ should be applied. **Reflectometric interference spectroscopy** is again more suitable for obtaining kinetic/affinity constants rather than for biosensing purposes.⁶⁵

Microcantilever-based biosensors are working in a label-free mode with a quick response time and ability to detect changes in the mass at the attogram (10^{-18} g) level.⁶⁶ The surface of one side of the cantilever is covered by a biorecognition element, while the other surface is passivated to resist any binding. When a recognition takes place a difference in a surface stress between these two surfaces results in a measurable mechanical deflection.⁶⁷ This method can detect a single cell or a single nanoparticle, but when it comes to analysis of proteins the techniques is not particularly sensitive with a detection limit down to sub-nM level.⁶⁶ Mechanical microcantilever sensing can be integrated into a microfluidic chip⁶⁶ or multiplexed analysis is possible,²⁸ but usefulness of this technique especially in analysis of various types of cancerous cells have to be proved yet.

Quenching of an intrinsic SWCNT fluorescence is a label-free method of analysis relying on modulating of fluorescence of SWCNTs by a flexible NTA-nickel tether attached to His₆-tagged lectins. After a glycoprotein is bound to lectin, a nickel ion moves away from the SWCNT surface, partially restoring quenched fluorescence of a SWCNT, which can be monitored in a real time, providing kinetic/affinity constants. The achieved detection limit is not that impressive (≈ 670 nM), but authors suggest that there is a room for improvement by using high quality nanotube sensors.^{68, 69} In a recent study authors extended this initial study for glycoprofiling of different forms of IgGs.⁷⁰

Label-based lectin biosensors

In this section only the most sensitive label-based lectin biosensors will be described or those ones with some advanced approaches provided.

Quantum dots (QDs), semiconductive nanoparticles exhibiting quantum mechanical properties⁷¹ utilisable in fluorescent and electrochemical sensing, were successfully applied in analysis of intact glycoproteins and various types of cells.

In a very interesting approach two types of QDs were employed for a simultaneous analysis of a biomarker of colorectal cancer CEA (a carcinoembryonic antigen) and a therapeutic drug C225 (cetuximab), applied for treatment of colorectal cancer.⁷² The surface with two immobilised lectins EEL (*Euonymus europaeus* lectin for binding of C225) and Con A lectin (for binding of CEA) were immobilised on the same surface and incubated with QDs

labelled proteins (i.e. CdSe/C225 and ZnO/CEA). In the next step, a sample containing both glycoproteins C225 and CEA displaced QDs labelled proteins from the surface. Thus, QDs released from the surface were subsequently detected in a single square wave voltammogram, since redox peaks for detection of Cd and Zn ions were well separated. A detection limit for CEA was 3 pM and for C225 of 230 nM, well below cut-off diagnostic values.⁷² Moreover, the authors suggested that it would be possible to detect simultaneously 5-6 analytes in a single run, as judged from the number of non-overlapping metal peaks.⁷²

A novel 3-D architecture was formed on the surface of a glassy carbon electrode by combining gold nanoparticles and nitrogen-doped carbon nanotubes with annexin V for selective attachment of apoptotic leukemic cells.⁷³ The cells were glycoprofiling by a CdTe QD-based nanoprobe containing lectins and the amount of nanoprobe attached to cells was quantified by an electrochemical stripping voltammetry. By this approach not only as low as 48 cells could be detected, but the method allowed to monitor a progression of apoptosis triggered by an apoptosis inducer.⁷³

A supersandwich strategy for a signal amplification was applied for an analysis of a CCRF-CEM cancerous cell line down to a concentration of 50 cells/mL.⁷⁴ The surface was modified by multi-walled carbon nanotubes (MWCNTs) loaded with gold nanoparticles and finally Con A was physisorbed to complete a biorecognition interface. After cells were incubated with this biosensor a supersandwich was formed by interaction of cells with a DNA concatamer loaded with CdTe QDs. A DNA concatamer was built up from a DNA aptamer recognising the cells, a signal DNA loaded with a QD and an auxiliary DNA to link a DNA aptamer with a few GD-labelled signal DNAs. Thus, a DNA concatamer contained a single recognising site (a DNA aptamer) and quite a few QDs attached. Detection of cancer cells was done by an anodic stripping voltammetry of Cd²⁺ ions. A direct comparison of a supersandwich biosensor configuration with a sandwich model formed by an incubation of the cells only with a DNA aptamer-QD conjugate showed enhancement of a current signal by a factor of 5.6.⁷⁴

The same cell line was analysed with the biosensor prepared from graphene loaded with dendrimers and Con A.⁷⁵ Subsequently, gold nanoparticles loaded with aptamer and HRP were incubated with the biosensor to generate electrochemical signal. This approach could detect as low as 10 cells/mL and to see dynamic differences in glycan composition after treatment with enzymes and small molecular inhibitors.⁷⁵

Redox labels are of particular interest to exploit various sensitive electrochemical techniques in biorecognition. A sandwich strategy was employed for analysis of a K562 leukemic cell line.⁷⁶ Con A lectin was immobilised on a mixed SAM layer and after cells were bound to this receptive layer, gold nanoparticles loaded with ferrocene moieties (redox labels) and Con A molecules were making a sandwich configuration. Current read from a cyclic voltammetry was then proportional to the cell concentration with a limit of detection down to 73 cells/mL.⁷⁶

A novel lectin biosensor based on off-electroluminescence was prepared on a graphite electrode modified by SWCNTs with attachment of a Ru complex and Con A lectin.⁷⁷

Upon binding of pathogenic *E. coli* O157:H7, the biosensor exhibited a decrease of an electroluminescent signal proportional to the cell concentration with a limit of detection of 127 cell/mL, a recovery index of 89-95% and an assay time of 70 min.⁷⁷ An on-electroluminescent approach was developed for a sandwich format of analysis.⁷⁸ A leukemic cell line K562 was attached on a surface patterned by MWCNTs and Con A. A sandwich configuration was completed, when cells were incubated with a hybrid nanoprobe containing Con A, Ru-silica nanoparticles and gold nanoparticles. Upon binding of this nanoprobe to the cells, an increase in electroluminescent signal was observed and cells could be detected down to a concentration of 600 cells/mL.⁷⁸ Moreover, dynamic changes in the glycan composition on the cell surface after treatment with inhibitors of glycan processing enzymes could be observed.⁷⁸

Hybrid nanoparticle/redox labels were utilised in some detection platforms allowing to introduce a large number of redox probes for an enhanced sensitivity of detection. A combination of carbon nanotubes and gold nanoparticles was employed for immobilisation of Con A lectin.⁷⁹ A nanoprobe consisting of carbon nanotubes, gold nanoparticles, thionine and mannose units was incubated with a lectin modified surface to form an electrochemically active complex due to presence of a redox thionine moiety. Such a complex was upon incubation with human lung cancer cells displaced from the surface, resulting in a decrease of current obtained by a differential pulse voltammetry. Two different cells lines 95-D and H1299 could be detected down to a concentration of 580 cells/mL and 12 cells/mL, respectively. Moreover, authors managed to calculate a number of mannose units present on the surface of a single cell.⁷⁹

Future prospects

Engineering of lectins and use of lectin-like recognition elements

There are few approaches, which can be applied in a biosensor construction with lectins and lectin-like molecules having advanced properties prepared from modified/engineered lectins and glycan-processing enzymes; DNA aptamers and lectin-like peptide aptamers.

Engineering of lectins with novel recognition profiles, high stability and high affinity will be an important issue to enhance practical usefulness of lectin-based biodevices. New lectins with novel binding preferences^{80, 81}, could be quite effectively produced by mutations of wild type lectins (i.e. site-directed mutagenesis for changed specificity of lectins^{82, 83} or by affecting valency of lectin binding^{84, 85}). Alternatively glycan binding proteins with improved specificity of biorecognition can be prepared by inactivation of glycosidases, while preserving their binding abilities.^{86, 87} Better understanding of glycan biorecognition by lectins can identify specific amino acids responsible for recognition of a particular mono- or oligosaccharides, a glycocodon theory^{88, 89}, can enhance success of preparation of lectins with higher selectivity of affinity of glycan binding. Moreover repertoire of lectins with novel properties can be further enhanced by their dimerisation⁹⁰, multimerisation⁹¹ or by attachment of boronic acid derivatives to lectins⁹².

DNA aptamers with lectin-like properties were recently developed in the Binghe Wang's lab⁹³, using an extending library of nucleotides modified by the incorporation of a boronic

acid moiety. Such DNA aptamers were able to detect glycoproteins with higher affinity compared to DNA aptamers produced only from a natural nucleotide library.⁹³ Moreover, DNA aptamers produced from a library of pyridine-derivatised nucleotides proved to be efficient in generation of high affinity DNA aptamers against novel ligands with a high affinity of binding⁹⁴ and such a strategy can be useful for development of a highly stable DNA aptamers against glycan moieties, as well. So far, DNA aptamers with glycan recognising properties have not been applied in preparation of biosensors applicable in glycoprofiling.

Lectin-like peptide aptamers have not been developed yet, but it is a question of time, when peptide aptamers with glycan recognising properties will be developed. Peptide aptamer is a combinatorial protein molecule having a variable peptide sequence, with an affinity for a given target protein, displayed on an inert, constant scaffold protein.⁹⁵ Peptide aptamers due to a small size, stable scaffold are working very effective even, when immobilised on surfaces^{96, 97} and thus lectin-like peptide aptamers, when developed, should provide an enhanced stability, selectivity and sensitivity of glycan detection.

Controlled immobilisation of lectins

The process of lectin immobilisation can be controlled either by preparation of recombinant lectins with special tags applied to control orientation of lectins with a binding site exposed to the solution phase or by tuning interfacial properties of lectin biosensors with control of a lectin density on the surface.

Controlled orientation of lectins on the surface is an important issue to be addressed for construction of highly robust lectin biosensors. In an initial study Mahal's lab proved that, a controlled orientation of lectins on the glass surface applied for preparation of lectin microarrays can lower detection limit considerably.⁹⁸ In this case recombinant lectins having a His₆-GST tag were uniformly immobilised on the surface offering a limit of detection of 12 ng/mL, contrary to a limit of detection of 10 µg/mL achieved by a lectin microarray without a control of an immobilisation process.⁹⁸ Alternatively, lectins with Fc-fused fragment could be applied for an oriented immobilisation of lectins on Protein A/G modified surfaces.⁹⁹ Usefulness of such recombinant lectins in construction of lectin biosensors has to be proved yet.

Controlled density of lectins on surfaces is other issue to be carefully addressed since it is well known that glycan recognition can be modulated to a high extent by their density.¹⁰⁰ In our recent work we tried to control density of lectins on the biosensor surface by tuning a density of a functional group applied for bioconjugation within a mixed self-assembled monolayer on gold by a ratio of two thiols applied for surface patterning^{24, 37, 38, 59}, but the influence of the lectin density on an analysis of intact glycoproteins and cells should be studied in more details.

Other expected advances in lectin biosensors

From all detection schemes discussed above it seems that especially electrochemical techniques can provide sensitivity of glycan detection using lectins greatly outperforming a

fluorescent lectin microarray. There are still some issues to be explored such as integration of lectin biosensors into a highly parallel form of analysis, microfluidic platforms of analysis and the potential of graphene to prepare robust lectin biosensors should be addressed, as well.

Addressed immobilisation of lectins within an array of electrodes is a prerequisite, when a multiplexed electrochemical analysis is required. A parallel analysis by lectin biosensors have not been described yet, but such integration should not be that problematic.^{101,102} There are in principle two ways how a redox triggered immobilisation of lectins could be carried out. The first one would rely on electrochemical release, by a negative voltage, of a thiolated mask covering electrode to which lectin should be immobilised¹⁰³ or by one of quite a few methods with activation of functional interfacial groups triggered electrochemically.^{104–107} Poly(dimethylsiloxane) would be most likely a preferred material for making microfluidic devices due to obvious advantages and compatibility with an electrochemical detection platform.¹⁰⁸

Graphene since its discovery by Geim and Novoselov¹⁰⁹ will be without any doubt more and more often applied for glycoprofiling of intact cells and proteins, when integrated into lectin biosensors. Graphene can be prepared in many different ways and its interfacial properties can be tuned quite effectively, when working with graphene oxide as a starting material. Some studies suggest that in order to preserve a native structure of lectin after being adsorbed on graphene, this interface should be prepared in a controlled way¹¹⁰ and that affinity of interaction of lectin with its analyte depends on the strength of interaction with the surface¹¹¹. Moreover, Joshi's group found out that lectin-glycoprotein recognition can be different if lectins are immobilised on a surface and soluble glycoproteins are interacting with lectins compared to situation, when the glycoproteins are immobilised and probed by soluble lectins.¹¹² Thus, to get sensitive and selective graphene-based and other lectin biosensors, surfaces have to be prepared with a special care.

Active lectin-based glycoprofiling as an attractive way for active glycoprofiling of bacterial species directly in environmental, food and clinical samples was introduced by Joseph Wang and colleagues.¹¹³ The approach was based on immobilisation of Con A lectin on a template-based gold/nickel/polyaniline/platinum microengine self-propelled by formation of oxygen bubbles from H_2O_2 as a fuel. The microengine device was able to selectively pick-up its analyte, i.e. *E. coli* cells, transport and release them by movement through a low pH environment. A recent study suggests that cargo can be released not only by a change in pH, but also by addition of a saccharide.¹¹⁴ The device was successfully tested in urine, drinking water, apple juice and seawater and can be loaded not only with bacteria, but with polymeric drug carrying spheres, as well, for a possible therapeutic action.¹¹³ A similar concept of a self-propelled microengine for isolation of microbial cells was introduced by the same group by changing of Con A for boronic acid containing film.¹¹⁵ It was suggested that a problematic aspect of using relatively high concentration of H_2O_2 as a fuel can be overcome by using microengines propelled by magnetic field¹¹⁶ or ultrasound¹¹⁷. Recently nanoengines were constructed in a way to reach cellular interior.¹¹⁸ It can be assumed, that there is a bright future for such devices for an active and autonomous glycoprofiling directly in a diverse range of environments or even within cells.

Conclusions

Lectin biosensors, especially electrochemically based-ones, already outperformed a lectin microarray technique in term of sensitivity provided. Moreover, some lectin biosensors could work in a label-free mode of operation and properties of traditionally applied gold surface for biosensor preparation could be tuned quite effectively by formation of mixed SAM layers with controlled immobilisation at nanoscale. For examples EIS is extremely sensitive detection techniques for analysis of glycoproteins with detection limits down to the fM-aM level (Tab. 2). Other label-free techniques could not outperform lectin microarray in terms of sensitivity and are not suitable for early stage diagnostics. Even though there is only one report on detection of glycoproteins by lectin biosensors operated in label-based mode (Tab. 3), label-free electrochemical approach seems to be more sensitive (Tab. 2). However, label-based electrochemical or electroluminescent techniques have been successfully applied in direct glycoprofiling of intact cells since after binding every single cell contain up to 10^{10} glycan moieties⁷⁹, resulting in a highly amplified signal and low limit of detection (i.e. 12 cells/mL)⁷⁹.

It is obvious that electrochemical label-free or label-based techniques are extremely sensitive and suitable for detection of low concentration of analytes. Additionally, electrochemically triggered immobilisation can be applied for immobilisation of lectins on an array of electrodes in a non-contact and automatic way. Contrary, lectin biosensors are still behind lectin microarrays, when throughput of analysis is compared. As highly parallel glycoprofiling by lectin biosensors as by lectin microarrays probably would not be possible, so it can be anticipated, that lectin biosensors are more suitable to be applied for diagnostic purposes, rather than for a preliminary glycoprofiling of a vast number of samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Lectins can detect a small change in the glycan composition. *Maackia amurensis* agglutinin (MAA-I) is specific for binding to α -2,3-linked sialic acid, while *Sambucus nigra* agglutinin (SNA-I) recognise α -2,6-linked sialic acid.

Fig. 2.

Typical label-free (i.e. lectin immobilised and a glycoprotein attached, left) and label-based sandwich (a labelled antibody or lectin is completing a sandwich) lectin biosensor configuration. Lectin is in both cases immobilised on SAM layer and then the surface is exposed to the solution containing glycoprotein and in case of a sandwich configuration, finally labelled antibody is injected.

Tab. 1

The most common lectin applied in preparation of lectin biosensors with a specificity of binding and other characteristics

Lectin	Specificity	M _w / SU	GP
Concanavalin A	αMan, αGlc	104 kDa/4 SU	No
SNA	NeuNAcα2-6Gal/GalNAc	140 kDa/4 SU	Yes
RCA I	Gal	120 kDa/2 SU	Yes
MAA II	NeuNAcα2-3Galβ1-4GalNAc	130 kDa/2 SU	Yes
WGA	GlcNAc	36 kDa/2 SU	No

Man: mannose, Glc: glucose, NeuNAc: N-acetylneuraminic (sialic) acid, Gal: galactose, GalNAc: N-acetylgalactosamine, GlcNAc: N-acetylglucosamine, M_w: molecular weight, SU: number of subunits, GP: glycoprotein

Tab. 2

Analytical parameters of label-free lectin biosensors

Method	Analyte	LR	DL	Ref.
EIS	Fet/ASF	15 fM-15 pM	15 fM	35
EIS	Fet/ASF	300 aM-3 nM	300 aM	38
EIS	Fet/ASF	0.5 aM-5 nM	0.5 aM	37
EIS	Serum, GPs	(aM) fM-nM *	low (aM) fM *	24
EIS	AFP	1 fM-100 fM	1 fM	39
Capacit.	IgG	33 nM-667 nM	33 nM	52
SPR	GPs	67 nM-6.7 μ M	67 nM	55
QCM	GPs	335 nM-6.7 μ M	67 nM	119
F of SWNT	glycoconjugate	670 nM-6.7 μ M	670 nM	68
<i>EIS</i>	<i>liver cancer cells</i>	$234 \cdot 10^6$ (1/mL)	234/ml	41
<i>SPR</i>	<i>E. coli O157:H7</i>	10^3 - 10^8 (1/mL)	10^3 /ml	56

LR: linear range, DL: detection limit, EIS: electrochemical impedance spectroscopy, Fet: fetuin, ASF: asialofetuin, GPs: glycoproteins

* depending on lectin applied and configuration used, AFP: α -fetoprotein, Capacit.: capacitance assay, IgG: immunoglobulin G, SPR: surface plasmon resonance, F of SWNT: quenching of fluorescence of single-walled carbon nanotubes

Tab. 3

Analytical parameters of label-based lectin biosensors

Method	Analyte	LR	DL	Ref.
SWV	CEA	3 pM-4 nM	3 pM	72
ASV	cancer cells	50-10 ⁶ (1/mL)	50/ml	74
Fc EC	leukemic cells	73-17 ⁸ (1/mL)	73/ml	76
ECL	<i>E. coli</i>	127-10 ⁶ (1/mL)	127/ml	77
ECL	leukemic cell line	600-10 ⁷ (1/mL)	600/ml	78
DPV	lung cancer cells 1	12-16 ⁸ (1/mL)	12/ml	79
DPV	lung cancer cells 2	580-10 ⁸ (1/mL)	580/ml	79

LR: linear range, DL: detection limit, SWV: square wave voltammetry, CEA: carcinoembryonic antigen, ASV: anodic stripping voltammetry, Fc EC: ferrocene electrochemistry, ECL: electrochemiluminescence, DPV: differential pulse voltammetry