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Systematic review on lectin-based electrochemical biosensors for clinically relevant carbohydrates and glycoconjugates

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ABSTRACT

Carbohydrates and glycoconjugates are involved in numerous natural and pathological metabolic processes, and the precise elucidation of their biochemical functions has been supported by smart technologies assembled with lectins, i.e., ubiquitous proteins of nonimmune origin with carbohydrate-specific domains. When lectins are anchored on suitable electrochemical transducers, sensitive and innovative bioanalytical tools (lectin-based biosensors) are produced, with the ability to screen target sugars at molecular levels. In addition to the remarkable electroanalytical sensitivity, these devices associate specificity, precision, stability, besides the possibility of miniaturization and portability, which are special features required for real-time and point-of-care measurements. The mentioned attributes can be improved by combining lectins with biocompatible 0–3D semiconductors derived from carbon, metal nanoparticles, polymers and their nanocomposites, or employing labeled biomolecules. This systematic review aims to substantiate and update information on the progress made with lectin-based biosensors designed for electroanalysis of clinically relevant carbohydrates and glycoconjugates (glycoproteins, pathogens and cancer biomarkers), highlighting their main detection principles and performance in highly complex biological milieus. Moreover, particular emphasis is given to the main advantages and limitations of the reported devices, as well as the new trends for the current demands. We believe that this review will support and encourage more cutting-edge research involving lectin-based electrochemical biosensors.

1. Introduction

Carbohydrates are optically active polyhydroxy aldehydes or ketones and their derivatives are considered “key-biomarkers” to understand a wide spectrum of natural biochemical reactions and abnormal metabolic processes [1]. They are actively present on whole cells and tissues, attached to lipids, and are expressed by almost all secretory proteins (hormones, enzymes, neurotransmitters, toxins and antibodies) in body fluids, after specific post-translational protein modification events – glycosylation [2,3]. In fact, the proper functioning of our reproductive, digestive and nervous systems, as well as drug-induced reactions used in the treatment of heart disorders, cancer and diabetes depend on correct glycosylation processes [3,4]. Glycoconjugates are also valuable coding information expressed on the outer surfaces of many viruses, bacteria, protozoa and fungi, but the physicochemical similarity of the sugar moieties complicates the identification of their molecular sequences and correlation with a given pathological process [5].

Site-selective carbohydrate binders are useful for pathogen recognition, disease diagnosis, development of therapeutic agents and vaccines [1,6,7]. Chronologically, the first findings on proteins with these characteristics were registered in the late 19th century, being known as hemagglutinins (or phytoagglutinins, since they were initially discovered in plant extracts) [8]. The hemagglutinating effect was originally described by Peter Hermann Stilmark [9]. This property was associated with a highly toxic hemagglutinin isolated from the seeds of castor bean plant (*Ricinus communis*), known as ricin [9,10]. It became quite popular ninety years later, after being used in the murder of the Bulgarian dissident writer Georgi Ivanov Markov, remembered as the "Umbrella

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Murder Mystery", which was motivated by political issues. Several other facts have marked the progress of science involving this class of proteins, including the isolation and purification of Concavalin A (ConA) from jack bean (*Canavalia ensiformis*) in its crystalline form, which was first carried out by James Batcheller Sumner in 1919 [9]. Until the 1950s, hemagglutinins received little attention because they were believed to be restricted to the Kingdom Plantae (today considered ubiquitous in nature), and their impressive abilities to recognize different blood groups (ABO system) or biomarkers on the cell surface were unknown [8]. With the discovery of blood type-specific hemagglutinins, the term "lectin" was proposed by William C. Boyd and Elizabeth Shapleigh in 1954 to define all carbohydrate-specific agglutinins of nonimmune origin, regardless of the source [11]. Different lectins from plants, microorganisms and animals were isolated in the following decades, resulting in important advances in different biotechnological segments, especially after further studies on their physicochemical properties, primary and three-dimensional structures, and recognition domains [8,9,12,13].

Currently, it is well-established that lectins are a relatively heterogeneous group of natural oligomeric proteins with similar sequences, which act as glycocode deciphers. They are able to recognize, distinguish and bind reversibly to free and bound carbohydrates, in addition to enabling the identification of new glycoproteins and their features [5]. These biomolecules are classified by their specificity for different monosaccharides (mannose, galactose/*N*-acetylgalactosamine, *N*-acetylglucosamine, fucose and *N*-acetylneuraminic acid), general structure (merolectins, holoectins, chimerolectins and superlectins) and family (legume lectins, monocot mannose-binding lectins, type II ribosome-inactivating proteins, among others) [14]. The interaction lectin-carbohydrate occurs with high affinity and specificity at particular protein sites through hydrogen bonds, van der Waals and hydrophobic interactions [10,15]. Additionally, some divalent metallic cations (e.g., Ca^{2+}, Mn^{2+} and Zn^{2+}) often mediate this process, forming positive bridges between oxygen atoms contained in carbohydrate structures and negatively charged protein residues [16]. Shallow binding sites on lectins tend to have lower selectivity for multiple carbohydrates, but this same feature makes it possible to study different target carbohydrates with a single protein [17,18]. Differences in the affinity of natural lectins or synthetic mimics of lectins also help define the architecture of their combining sites and functional groups that bind to carbohydrates [19]. The strength of the lectin-carbohydrate interaction increases as the dissociation equilibrium constant (K_d) decreases, so that the binding affinity is typically weaker if K_d <1.0 μmol L^{-1} [20]. The biorecognition ability of these proteins arises primarily from the conservation of the three-dimensional structure of their binding sites at the amino acid level, although there are other important physicochemical parameters to be considered (e.g., pH, pI, temperature, metal ion cofactor, balance of electrostatic forces and steric repulsion) [10,21–23]. Strict control of these conditions allows lectins to assume numerous exciting functions and applications.

Innovative and bold bioanalytical strategies to monitor carbohydrates and glycoconjugates in real time are among the noblest applications of these proteins. This task is performed masterfully by lectin-based biosensors, which are extremely sensitive and cutting-edge analytical tools, engineered with lectins immobilized on suitable optical or electrical transducers [24]. Devices that operate under electrochemical impulse are of current and broad interest, and this review addresses inherent progresses on these tools, i.e., lectin-based biosensors designed for electroanalysis of clinically relevant carbohydrates and glycoconjugates (glycoproteins, pathogens and cancer biomarkers), emphasizing their architectures, detection principle and performance achieved in highly complex biological milieus. General aspects of lectin biosensors were partially explored in previous reviews [5,15,24], but this proposal fills an important gap by gathering and systematizing the latest information (2015–2020) on lectin-based biosensors that operate under electrochemical stimulation. Over time, these platforms have been innovated by the interconnection of lectins with various semiconductors and biomolecules, leading to improvements in functioning principles and detection capabilities not seen before, either with or without labels. The literature search focused on reports published in peer-reviewed journals indexed in Web of Science, ScienceDirect, Scopus and PubMed databases, and available in English. The following keywords were used for searching: lectins, electrochemical biosensors, carbohydrates, glycoconjugates, detection, quantification, and clinical treatment. A total of forty-two research articles were identified and reviewed in order to critically address issues on device development, detection mechanisms, signal enhancement, measurement accuracy, miniaturization protocols, and highlighting the trends and challenges for analysing carbohydrates and glycoconjugates in real samples.

2. Lectin-based electrochemical biosensors

Detection of carbohydrates chains by sophisticated lectin-based electrochemical biosensors is becoming increasingly prominent due to their inherent characteristics. Such devices introduced novel concepts for biorecognition assays based on molecular interactions between the target analyte and the biosensor surface, which are subsequently transduced into an appropriate electrochemical signal. The architecture of electrochemical lectin-based biosensors is diverse and it is being continuously improved through the combination with micro- and nanoscale 0–3D semiconductors (carbon allotropes, metallic nanoparticles, polymers, metallic complexes, (in)organic-(in)organic nanocomposites, among others), which significantly improve the sensitivity and the overall performance. These materials feature high surface area, chemical stability and biocompatibility, and provide a significant improvement in charge transport for biosensing applications [24–26].

The interaction and detection processes of glycans occur by affinity in the molecular templates contained in the structure of immobilized lectins that, in turn, recognize the expression of the target molecules through N- and O-linkages. The device architecture defines the glycan sensing detection scheme, i.e., based on (1) lectin directly immobilized on solid supports, (2) lectin anchored on glycoprotein-modified supports, and (3) sandwich models involving lectin-lectin and lectin-antibody or DNA aptamer pairs [5,24]. Fig. 1 shows lectin-based biosensors with different configurations, along with the most targeted sugars moieties. In general, chemisorption techniques, covalent bond formation, cross-linking reagents and entrapment in a polymer network are the most commonly used strategies for protein immobilization [24]. For detection, many systems require labeled analytes, but label-free signal generation is also among the fantastic abilities of these devices, which simplifies the identification of glycan sequences [5]. Still, label-free electrochemical tools are prone to suffer from non-specific adsorption, which has been investigated and often solved by the scientific community in various ways [27–29]. In addition, some prototype biosensors track the lectin-carbohydrate interaction in a reversible and continuous way, eliminating more laborious experiments and undesirable chemical regeneration steps [4]. These attributes are also useful for understanding and correlating physiological and pathological processes triggered when such interactions occur in living organisms. It is known that there are other synthetic (e.g., metal nanoparticles and complexes, boron-containing compounds, and polymers) and biological agents (e.g., enzymes, peptides, liposomes, dendrimers, antibodies, DNA and RNA aptamers) used in specific recognition of carbohydrates and glycoconjugates, but attention to lectins has increased dramatically with the evolution of recombinant strategies to tailor or enhance their binding characteristics [30,31].

Quantitative or semi-quantitative electroanalytical measurements are often performed by voltammetry (the current is measured as dependent variable of the applied potential, which changes continuously or stepped over time), chronoamperometry (the current is measured at a fixed potential over time) and electrochemical impedance spectroscopy (the charge-transfer resistance is measured during

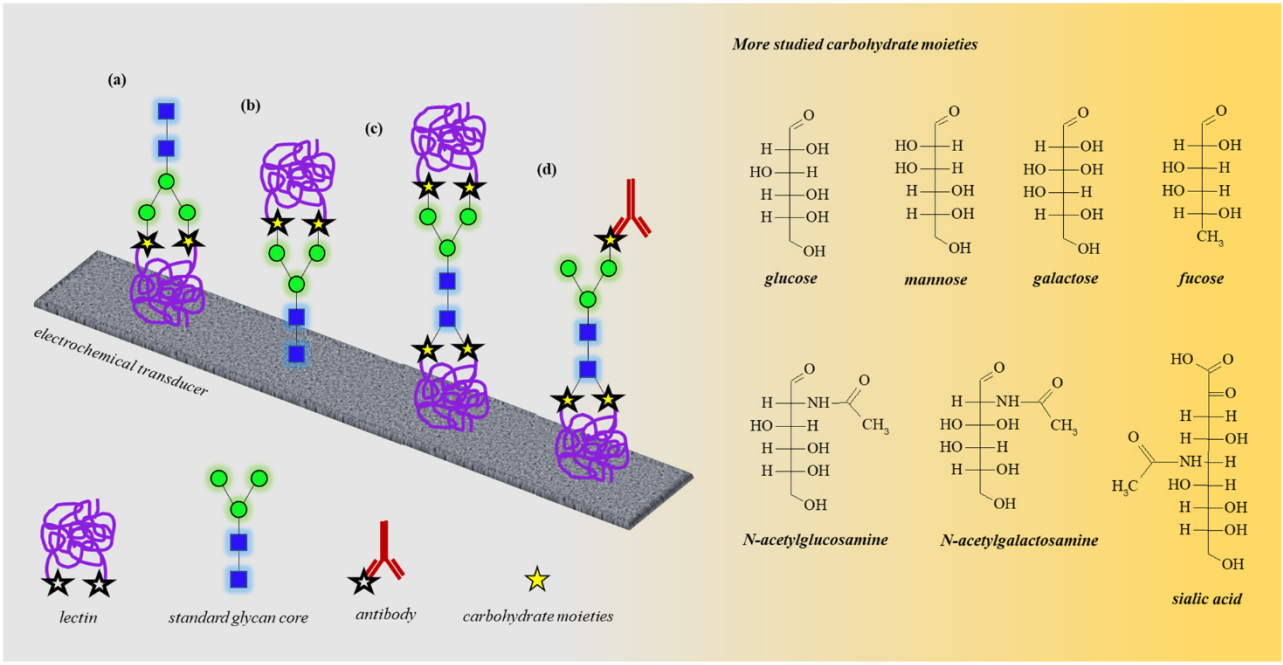

Fig. 1. Analog architectures and detection principles of biosensors developed with lectin **(a)** directly immobilized on solid supports, **(b)** anchored on glycoprotein-modified supports, and sandwich models involving **(c)** lectin-lectin and **(d)** lectin-antibody pairs. Fisher's projections for some of the most investigated target sugars are shown on the right.

perturbations of electrical double-layer formed on the conductive surface, caused by alternating current amplitudes) [4,5,24], which provide accurate, fast and high-throughput measurements for a given redox reaction. Moreover, electroanalytical methods for carbohydrates conducted with lectin-based biosensors display excellent sensitivity, reaching femto-aptomolar levels, thus allowing a better understanding of highly complex physiological and pathological events involving these analytes. More information on this subject can also be found in Silva [32], Kumar and Kalkal [33]. There are other efficient methods based on mass spectrometry [34], affinity chromatography [35], capillary electrophoresis [36] and immunoenzymatic assays [31,37], but electroanalytical procedures can be more advantageous when considering cost, ease of operation, portability and response time.

2.1. Free carbohydrates

Electrochemical detection of carbohydrates is classically based on redox reactions involving glycoenzymes, especially glucose oxidase (GOx) [26,38]. Some challenges regarding the strict control of immobilization conditions in order to maintain and maximize enzyme activity still persist [26]. There are reports in which the detection of oligo- and poly-saccharides is unfeasible without their (bio)chemical cleavage into simpler sugar units and/or previous steps for separation and extraction of the analytes, increasing the experiment time, changing the nature of the target molecule, and making impossible direct electroanalysis [26,39]. Therefore, lectin-based biosensors are being increasingly designed (Table 1) since they overcome the constraints mentioned [40].

ConA lectin and its biocomposites associated with carbon-derived transducers arise among the most investigated strategies [41–45], as indicated in the first section of Table 1. Yao et al. [41] used ConA and GOx as building blocks to assemble layer-by-layer films on a poly (diallyldimethylammonium)-modified pyrolytic graphite for stimuli-responsive bioelectrocatalysis of the proposed biosensor towards glucose oxidation. The films showed pH-sensitive on-off properties in the presence of the electroactive probe ferrocenedicarboxylic acid, leading to greater reactivity at pH = 4. Operating a device with five protein bilayers by chronoamperometry, it was possible to quantify glucose within a linear range (5.0×10^{-4} - 5.0×10^{-3} mol L^{-1}) that proves the efficiency of the biorecognition scheme for the development of new electrochemical biosensors for carbohydrates. Ortiz et al. [43] proposed an innovative nanoarchitecture based on the non-covalent functionalization of multi-walled carbon nanotubes (MWCNT) with ConA, which presents site-specific supramolecular binding capacity for glycobiomolecules. After drop-casting a glassy carbon electrode (GCE) with a MWCNT-ConA dispersion, two glucose biosensors were prepared with GOx or GOx-horseradish peroxidase (HRP), as outlined in Fig. 2. The bienzymatic biosensor GCE/MWCNT-ConA/GOx-HRP had remarkedly superior performance than the monoenzymatic biosensor, i.e., it operated within a linear range with two orders of magnitude (2.0×10^{-6}- 4.1×10^{-4} mol L^{-1}), besides achieving a 5.2 times higher sensitivity and a detection limit almost one order of magnitude lower (LOD; estimated at 0.31 μmol L^{-1}). The bienzymatic biosensing signal was negatively influenced by the co-existence of uric (76% for 2.5×10^{-5} mol L^{-1}) and ascorbic acid (23% for 5.0×10^{-6} mol L^{-1}) due to the high working potential, but the competitive analytical characteristics were significantly improved by using a Nafion® conductive membrane, decreasing the interferences to 22% and 4.5% for uric and ascorbic acid, respectively.

Alternative non-enzymatic electrochemical biosensors to determine free carbohydrates can also be constructed with lectins, as reported by Danielson et al. [46]. These researchers proposed a highly sensitive field-effect transistor device based on graphene decorated with gold nanoparticles (AuNPs) for lactose detection in a liquid-gate measurement configuration. The biorecognition principle was mediated by the mutant human galectin-3 protein (h-Gal-3 M249C) previously immobilized on AuNPs/graphene, which specifically binds to β-galactoside residues. Under optimized conditions, h-Gal-3 M249C/AuNPs/graphene-based biosensor achieved an effective response to lactose from 1.0×10^{-15} to 5.0×10^{-12} mol L^{-1} (LOD = 2.0×10^{-16} mol L^{-1}). Such efficiency was maintained even in the presence of $Lewis^X$, i.e., a trisaccharide composed of fructose and *N*-acetyllactosamine, considered one of the most important blood group antigens with a critical role in cell-to-cell recognition and cancer metastasis. Qin et al. [45] analyzed the effectiveness of ConA

Table 1
Configuration and analytical performance of different lectin-based electrochemical biosensors proposed (2015–2020) for detecting carbohydrates and glycoconjugates.

Analyte	Lectin	Biosensor configuration	Detection technique	Linear range	LOD	Real sample	Ref.
Free carbohydrates							
D-glucose	ConA	pyrolytic graphite/ConA and glucose oxidase composite film	chronoamperometry	5.0×10^{-4} - 5.0×10^{-3} mol L^{-1}	–	–	[41]
D-mannose, N-acetyl-*D*-glucosamine, *L*-fucose, *D*-galactose	WGA, SBA, PNA, UEA-I or ConA	gold sensor/polydopamine SAM/perfluorophenylazide conjugated to the target carbohydrate/specific lectin	EQCM	–	–	–	[42]
D-glucose	ConA	GCE/MWCNT-ConA/ Glucose oxidase GCE/MWCNT-ConA/ horseradish peroxidase conjugated with glucose oxidase	chronoamperometry	5.0×10^{-6} - 1.2×10^{-3} mol L^{-1} 2.0×10^{-6} - 4.1×10^{-4} mol L^{-1}	1.6×10^{-6} mol L^{-1} 3.1×10^{-7} mol L^{-1}	human serum	[43]
Sialyl-Lewis X	E-selectin	gold sensor/IgG Fc-binding domain conjugated with E-selectin	EQCM	~ 2.0–142.2 μg mL^{-1}	1.3 μg mL^{-1}	–	[44]
Lactose	M249C hGal-3	Si–SiO_2 substrate/ graphene/gold nanoparticles/ M249C hGal-3	FET	1.0×10^{-15} - 5.0×10^{-12} mol L^{-1}	2.0×10^{-16} mol L^{-1}	–	[46]
Mannan	ConA	GCE/Nafion®/carbon nanospheres/ConA GCE/Nafion®/graphene/ gold nanoparticles/ alkanethiol/ ConA	EIS	1.0×10^{-9} - 5.0×10^{-7} mol L^{-1}	8.0×10^{-11} mol L^{-1} 3.0×10^{-11} mol L^{-1}	–	[45]
Glycoproteins							
human IgG	SNA	gold electrode/sulfobeatine /SNA	EIS	1.0×10^{-17} - 1.0×10^{-12} mol L^{-1}	1.0×10^{-16} mol L^{-1}	human serum from healthy and systemic sclerosis patients	[50]
human IgG	RCA-I	gold electrode (array chip)/ SAMs of zwitterions/RCA-I	EIS	1.0×10^{-17} - 1.0×10^{-12} mol L^{-1}	–	human serum from healthy, seronegative, and seropositive rheumatoid arthritis patients	[51]
invertase	ConA	GCE/reduced graphene oxide/ConA	EIS	1.0×10^{-14}- 1.0×10^{-8} mol L^{-1}	–	–	[49]
human IgA	AIA	n-type SiO_2 substrate/ aminosilane conjugated with glutaraldehyde/AIA	FET	1.0×10^{-1} - 1.0×10^{2} μg mL^{-1}	1.0 μg mL^{-1}	human sweat from healthy individuals	[52]
Pathogens							
Dengue type 2 Zika Chikungunya Yellow fever virus	ConA	gold electrode/cysteine/ zinc oxide nanoparticles/ ConA	EIS	3.6×10^{-2} - 1.8×10^{-1} PFU mL^{-1}	3.82×10^{-2} PFU mL^{-1} 4.21×10^{-2} PFU mL^{-1} 6.2×10^{-2} PFU mL^{-1} 4.37×10^{-2} PFU mL^{-1}	human serum from healthy and arbovirus-infected patients	[53]
H9N2 Influenza type A	PNA	gold screen-printed electrode/nanocomposites of graphene and gold nanoparticles/Fetuin A treated with neuraminidase/PNA	EIS	–	1.0×10^{-8} U mL^{-1}	allantoic fluid from chicken embryonated eggs	[57]
Escherichia coli	ConA	gold electrode/monolayer of alkanethiol and dithiothreitol/ConA	EIS	–	7.5×10^{1} cells mL^{-1}	–	[67]
Escherichia coli	ConA	GCE/graphene–Nafion® /polyaniline SAM/ glutaraldehyde/ConA	EIS	–	4.3×10^{1} cells mL^{-1}	–	[66]
Escherichia coli	ConA	Pyrolytic graphite electrode/ConA carbon screen-printed electrode/ConA	EIS	1.0×10^{3} - 1.0×10^{5} CFU mL^{-1} 1.0×10^{3} - 1.0×10^{6} CFU mL^{-1}	3.0×10^{3} CFU mL^{-1} 1.9×10^{3} CFU mL^{-1}	bacterial cultures from sewage sludge	[68]
O157:H7 *Escherichia coli* strain	WGA	gold electrode/amine reactive crosslinker SAM/ streptavidin/Anti-*E. coli* biotin-antibodies/WGA	EIS	1.0×10^{2} – 1.0×10^{6} CFU mL^{-1}	1.0×10^{2} CFU mL^{-1}	–	[62]
Candida albicans, Candida krusei, Candida	ConA or WGA	gold electrode/cysteine SAM/gold nanoparticles	EIS			*Candida* spp. clinical isolates	[70]

(*continued on next page*)

Table 1 (*continued*)

Analyte	Lectin	Biosensor configuration	Detection technique	Linear range	LOD	Real sample	Ref.
tropicalis, and *Candida parapsilosis*		coated with carboxythiol/ ConA or WGA		1.0×10^2 – 1.0×10^6 CFU mL^{-1} for both lectins	1.0×10^2 CFU mL^{-1} for both lectins		
Cancer biomarkers							
α-2,6-sialylated glycans	SNA	GCE/reduced graphene oxide-aliphatic amines-ionic liquid nanocomposite/ gold–platinum alloy nanoparticles/SNA	EIS	1.0×10^1 - 1.0×10^6 fg mL^{-1} 1.0×10^6 - 1.0×10^9 fg mL^{-1}	3.0 fg mL^{-1}	human serum spiked with the analyte	[72]
α-2,6-Sialylated glycans	SNA	GCE/gold-platinum-polypyrrole nanocomposite/SNA/ Glucose oxidase	chronoamperometry	1.0×10^1 - 8.0×10^2 ng mL^{-1}	3.0 fg mL^{-1}	human serum spiked with the analyte	[73]
α-2,3- and α-2,6-sialylated glycans	MAL and SNA	GCE/MWCNT coated with polyamidoamine dendrimer/diisocyanate crosslinker/MAL or SNA	DPV	1.0×10^1 - 1.0×10^3 fg mL^{-1} and 1.0×10^3 - 5.0×10^6 fg mL^{-1} for both analytes	3.0 fg mL^{-1} for both analytes	human serum spiked with the analytes	[75]
α-2,3-Sialic acid	MAL	GCE/MWCNT coated with polyamidoamine dendrimer/PDITC cross linker/MAL	DPV	1.0×10^1 - 1.0×10^3 fg mL^{-1}, and 1.0×10^3 - 1.0×10^6 fg mL^{-1}	3.0 fg mL^{-1}	human serum spiked with the analyte	[76]
α-2,3-Sialylated glycans	MAL	GCE/ palladium–platinum bimetallic alloy nanocrystals/ thiol-containing boronic acid/ nanocomposite of gold coated with MAL and poly (methylene blue)	DPV	1.0×10^1 - 1.0×10^8 fg mL^{-1}	3.0 fg mL^{-1}	human serum spiked with the analyte	[77]
α-2,6-Sialylated glycans	biotinylated SNA	GCE/gold nanorods-streptavitin nanocomposite/ biotinylated SNA/single-walled carbon nanohorns/ sulfur-doped platinum nanocluster and 3-amino-phenylboronic acid	DPV chronoamperometry	5.0–5.0×10^3 ng mL^{-1} 1.0–1.0×10^8 fg mL^{-1}	5.0×10^{-1} ng mL^{-1} 6.9×10^{-1} fg mL^{-1}	human serum spiked with the analyte	[78]
sialic acid	SNA	GCE/silver nanoflower incorporated with bovine serum albumin/SNA	EIS	1.35×10^2 – 1.35×10^7 cells mL^{-1}	4.0×10^1 cells mL^{-1}	–	[79]
PSA	SNA	gold electrode/alkanethiols SAM/anti-PSA antibody/ SNA or MAA	EIS	1.0×10^2 - 1.0×10^{12} ag mL^{-1}	1.0×10^2 ag mL^{-1}	human serum from healthy and prostate cancer patients	[74]
	MAA			–	–		
PSA and PSA glycans	MAA or SNA	polycrystalline gold electrode/alkanethiols SAM/(MAA or SNA) or anti-PSA antibody/SNA	EIS	–	4.0 amol L^{-1} or 5.0 amol L^{-1} for SNA-based biosensor depending on the SAM composition 4.0 amol L^{-1} for SNA immuno-based biosensor	human serum from a healthy donor	[80]
PSA glycans	MAA, LTA or SNA	gold electrode/alkanethiol SAM/Anti-PSA antibody/ lectin (MAA, LTA or SNA)	EIS	–	–	–	[81]
PSA	SNA	polycrystalline gold electrode/alkanethiols SAM/single-chain anti-PSA antibody fragment/SNA	EIS	1.0×10^{-1} - 1.0×10^2 ng mL^{-1}	1.0×10^{-2} ng mL^{-1}	–	[82]
PSA	SNA	gold electrode/zwitterion SAM/SNA/gold nanoshells with magnetic core coated with anti-PSA antibody	EIS	1.0×10^{-2} - 1.0 pg mL^{-1}	3.4×10^{-2} pg mL^{-1}	human serum spiked with the analyte	[83]
CEA	ConA	glass substrate/3D graphene foam/ polydopamine SAM/anti-CEA antibody labeled with ConA and horseradish peroxidase	DPV	8.0–1.2×10^2 ng mL^{-1}	~ 9.0×10^1 pg mL^{-1}	–	[84]
CEA	ConA	screen-printed carbon electrode/cysteamine self-assembled gold nanoparticles/ConA, WGA, or LCA	chronoamperometry	1.0–1.0×10^1 ng mL^{-1}	3.0×10^{-2} ng mL^{-1}	human serum from healthy and liver cancer patients	[86]
	WGA			1.0–1.0×10^1 ng mL^{-1}	5.0×10^{-2} ng mL^{-1}		
	LCA						

(*continued on next page*)

Table 1 (*continued*)

Analyte	Lectin	Biosensor configuration	Detection technique	Linear range	LOD	Real sample	Ref.
				5.0×10^{-1} - 7.0 ng mL^{-1}	1.0×10^{-2} ng mL^{-1}		
CEA	ConA	gold electrode/CEA aptamer/ConA conjugated with horseradish peroxidase	DPV	5.0–4.0 $\times 10^{1}$ ng mL^{-1}	3.4 ng mL^{-1}	human serum spiked with the analyte	[89]
A549 lung cancer cells	ConA	GCE/MWCNT/gold nanoparticles coated with thiomannosyl/MWCNT coated with Horseradish peroxidase and ConA	DPV	2.0×10^{1} - 4.0×10^{5} cells mL^{-1}	1.0×10^{1} cells mL^{-1}	–	[71]
QGY-7703 liver cancer cells				1.0×10^{2} - 1.0×10^{6} cells mL^{-1}	4.0×10^{1} cells mL^{-1}		
LNCaP prostate cancer cells				4.0×10^{1} - 1.0×10^{6} cells mL^{-1}	1.5×10^{1} cells mL^{-1}		
QGY-7701 liver cancer cells	ConA	GCE modified with thionine-bridged multiwalled carbon nanotube/gold nanoparticle composite/ConA labeled with horseradish peroxidase	DPV	5.0×10^{1} - 5.0 10^{6} cells mL^{-1}	2.0×10^{1} cells mL^{-1}	–	[85]
LNCaP prostate cancer cells				1.0×10^{2} - 1.0×10^{7} cells mL^{-1}	3.5×10^{1} cells mL^{-1}		
NHSF normal human skin-fibroblast cells, T47D highly invasive breast cancer cells, and MCF7 weakly invasive breast cancer cells	ConA and WGA	TiO_2 butterfly-like membrane nanostructures/ gold nanoparticles/ cysteine/ConA or WGA	EIS	1.0×10^{1} - 1.0×10^{6} cells mL^{-1} for both lectins-based biosensors and all cell lines	1.0×10^{2} cells mL^{-1} for WGA-based biosensor and MCF7 cells, and 1.0×10^{1} cells mL^{-1} for all other devices and cells	–	[87]
human breast cancer cells	ConA	gold electrode/WC10 cysteine-terminated peptide/gold nanoparticles coated with ConA/sodium alginate conjugated with glucose oxidase	EIS	5.0×10^{2} - 5.0×10^{5} cells mL^{-1}	1.5×10^{2} cells mL^{-1}	cultures of MCF-7 cancer cells	[88]
PC-3 prostate cancer cells	WGA	GCE/graphene oxide/ antibody/WGA	ECL	7.0×10^{2} – 3.0×10^{4} cells mL^{-1}	2.6×10^{2} cells mL^{-1}	–	[90]
PSA				5.0×10^{-1} - 4.0×10^{2} ng mL^{-1}	1.0×10^{-1} ng mL^{-1}		
fetuin	Cramoll 1,4	CGE/poly-L-lysine SAM/ carboxylated carbon nanotubes/Cramoll 1,4	SWV	0.5–2.5 $\times 10^{1}$ μg mL^{-1}	1.7 $\times 10^{-2}$ μg mL^{-1}	human serum from prostate cancer and benign prostatic hyperplasia patients	[92]
AFP	B-LCA	GCE/chitosan–graphene oxide/Anti-AFP antibody/ silver nanoparticles coated with B-LCA	SWV	2.5×10^{1} - 1.5×10^{4} pg mL^{-1}	1.2×10^{1} pg mL^{-1}	human serum spiked with the analyte	[93]
Galactosyltransferase (indirect detection as enzymatic activity)	AIA	gold electrode/bovine serum albumin conjugated with N-acetylglucosamine/ galactose/ gold nanorods coated with AIA and xanthine oxidase	ECL	1.0×10^{-3} - 1.0×10^{-1} U mL^{-1}	9.0×10^{-4} U mL^{-1}	lysates of HeLa human cervical cancer cells, CCRF-CEM human leukemic lymphoblasts cells, SMMC-7721 human hepatocellular carcinoma cells, and HL-7702 human hepatocyte cells	[94]
Tn antigen	VVA	gold screen-printed electrode/alkanethiol/VVA	EIS	2.5×10^{-2} - 5.0 μg mL^{-1}	6.8×10^{-1} μg mL^{-1}	human serum from healthy and cancer patients	[95]
T antigen	PNA	screen-printed gold electrode/akanethiols SAM/PNA	EIS	0 – 1.50 $\times 10^{2}$ μg mL^{-1}	1.16 $\times 10^{1}$ μg mL^{-1}	human serum from healthy and cancer patients	[97]

Lectin from Canavalia ensiformis (ConA), wheat germ (WGA), soybean (SBA), peanut (PNA), Ulex europaeus - type I (UEA-I), human E-selectin (E-selectin), Sambucus nigra (SNA), Ricinus communis - type I (RCA-I), Artocarpus heterophyllus (AIA), Maackia amurensis (MAA), Lens culinaris (LCA), biotinylated LCA (B-LCA), Cratylia mollis - isoforms 1 and 4 (Cramoll 1,4), Maackia amurensis (MAL), Lotus tetragonolobus (LTA), Vicia villosa (VVA), and mutant human galectin 3 (M249C hGal-3). Other abbreviations: carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), alpha-fetoprotein-L3 (AFP), glassy carbon electrode (GCE), multi-walled carbon nanotubes (MWCNT), self-assembled monolayer (SAM), electrochemical quartz crystal microbalance (EQCM), field-effect transistor (FET), electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), electrochemiluminescence (ECL), square-wave voltammetry (SWV).

immobilized on graphene or carbon nanospheres on the sensitivity of a label-free electrochemical impedimetric biosensor for mannan. It was proven that GCE/Nafion®/graphene/AuNPs/alkanethiol/ConA was the platform that offered the best sensitivity to monitor charge-transfer resistance (R_{ct}) changes as the concentration of mannan increased (1.0×10^{-9} - 5.0×10^{-7} mol L^{-1}; LOD = 3.0×10^{-11} mol L^{-1}). However, long-term storage of this biosensor still needs additional improvements, since the response to 1.0×10^{-9} mol L^{-1} mannan reduced by 9.5% and 12.4% after 10 days and 20 days, respectively.

2.2. Glycoproteins

Glycoproteins are proteins anchored to sugar residues through covalent bonds [47]. They are often present on the cell surface,

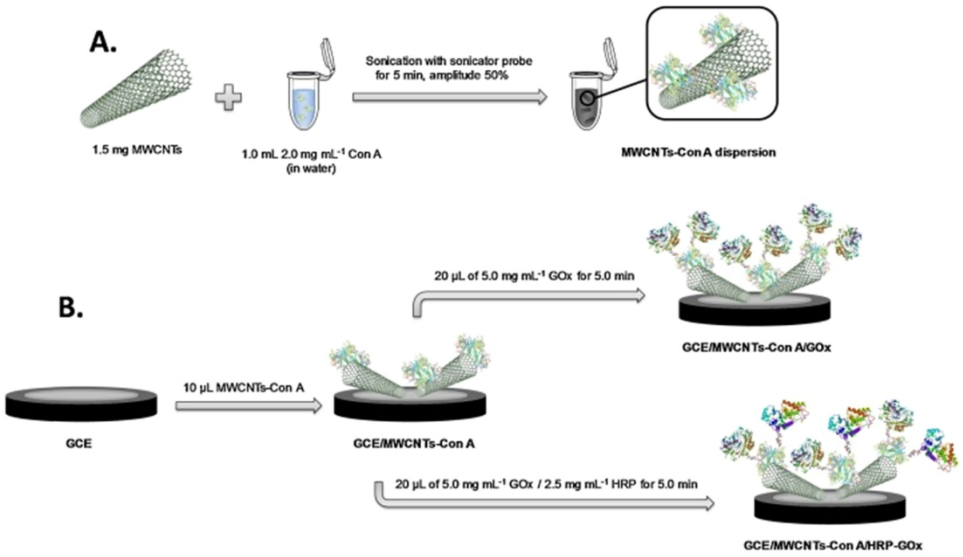

Fig. 2. Steps involved in preparing a glucose biosensor using a glassy carbon electrode modified with a MWCNTs-ConA dispersion. Adapted from Ortiz et al. [43] with permission from Elsevier.

participating in cell-cell interactions, infections caused by bacteria and viruses, and cell stress mechanisms [48]. The imperfections in glycoprotein glycosylation deserve special attention, as they are associated with several types of autoimmune diseases, so they need to be monitored insightful [47–49]. Therefore, lectin-based electrochemical biosensors for screening different glycoproteins (i.e., human IgG and IgA, and invertase) related with stress and autoimmune diseases have been reported (Table 1).

Systemic sclerosis is a chronic and progressive pathology that affects connective tissues and can debilitate virtually every organ in the body [50]. Serum autoantibodies are very useful for the early diagnosis of this pathology, but the analysis is quite challenging because their concentrations vary among patients who, in turn, may also have other autoimmune diseases. Klukova et al. [50] researched alternative *Sambucus nigra* lectin (SNA)-based protocols for glycoprofiling serum samples from healthy and systemic sclerosis patients. They tested and compared three different bioanalytical procedures using (i) enzyme-linked lectin assay with enzyme-labeled SNA, (ii) fluorescent SNA microarrays, and (iii) impedimetric SNA-based biosensor (Fig. 3). The electrochemical device was developed from the immobilization of SNA on sulfobetaine-modified gold electrode, which was used for biorecognizing the sialic acid expressed during human immunoglobulin (IgG) glycosylation processes. Within the several tested approaches, it showed the best analytical performance in terms of dynamic range (1.0×10^{-17} - 1.0×10^{-12} mol L^{-1}) and sensitivity (LOD < 1.0×10^{-17} mol L^{-1}), while also being simpler due to its label-free detection

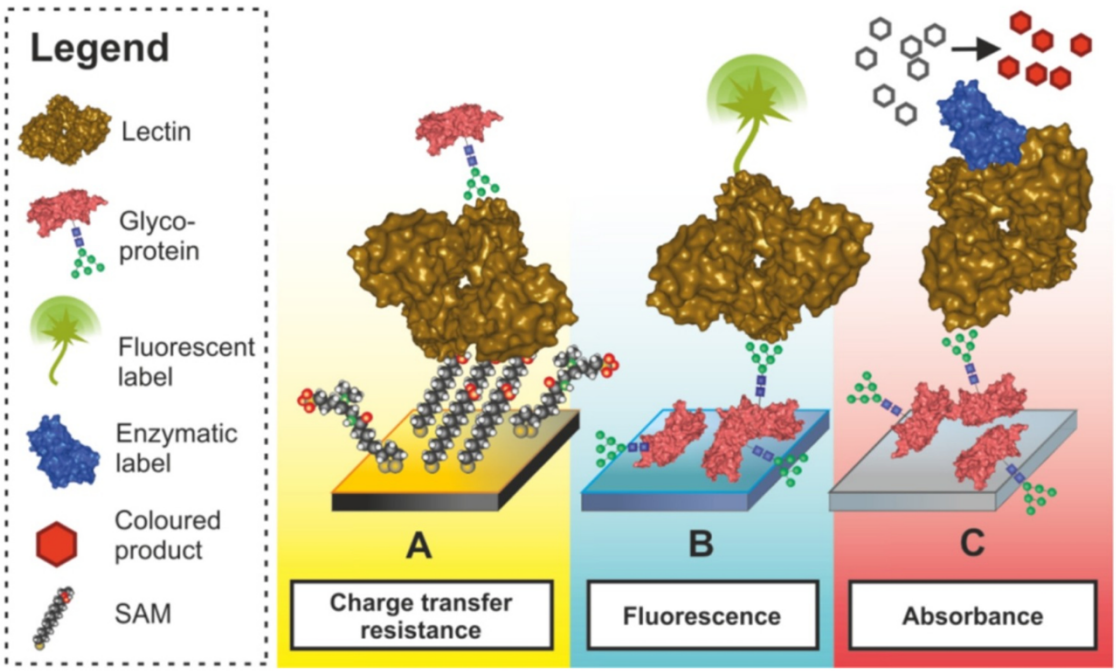

Fig. 3. General scheme applied in glycoprofiling of human serum samples with (A) lectin-based electrochemical impedance spectroscopy, (B) microarray with fluorescently labeled lectin, and (C) enzyme-linked lectin assay with enzyme-labeled lectin.
Adapted from Klukova et al. [50] with permission from Elsevier.

capacity. In contrast, the impedimetric SNA-based biosensor displayed lower reproducibility and no multiplexed ability.

Bertok et al. [51] managed to develop an impedimetric lectin-based eight-gold electrode microarray to confirm the presence of desialylated and degalactosylated IgGs during rheumatoid arthritis progression. The biosensing platform was built from a mixed self-assembled monolayer (SAM) consisting of two thiolated zwitterionic compounds (carboxybetaine and sulfobetaine) to reduce nonspecific interactions and allow covalent immobilization of *Ricinus communis* agglutinin I (RCA-I; a lectin that interacts specifically with galactose and *N*-acetylgalactosamine residues). The results suggested that RCA-I maintains its activity and ability to distinguish between different glycoforms, namely fetuin, asialofetuin, and IgGs from human serum. The most sensitive response was observed for asialofetuin (1.0×10^{-5} - 1.0×10^{-1} g mL^{-1}). EIS analysis agreed very well with the DAS28 index (rheumatoid arthritis disease activity score 28), indicating that this electrochemical procedure could be used to identify glycosylation changes in human IgGs related to rheumatoid arthritis.

Depression-related suicide currently affects 3–5% of the world's population, and it is believed that this mental illness will be the most prevalent by 2030 [52]. Knowing that secretory immunoglobulin A (s-IgA) is a stress marker present in biological fluids such as saliva and sweat, Hayashi et al. [52] studied a non-invasive detection method to measure s-IgA in human sweat, using a field effect transistor (FET) biosensor structured with *Artocarpus heterophyllus* lectin (AIA) immobilized on silane-modified SiO_2 substrate. AIA has four glycan-binding sites, allowing the as-prepared FET biosensor detect s-IgA at concentrations ranging from 0.1 to 100 μg mL^{-1}. The main difficulty reported for direct application of this method was its susceptibility to interference from agglutinated sweat components, sebum, bacteria, and mucin, which were non-specifically adsorbed on the electroactive surface. However, this limitation was eliminated after filtering the samples, allowing the use of AIA-based FET biosensor to daily monitor stress.

2.3. Pathogens

Glycoconjugates can also be found on the outermost layers of viruses, bacteria and fungi, so lectin-based biosensors are very useful for screening them, either qualitatively or quantitatively, as can be seen throughout the third section of Table 1. For example, lectins like ConA [53,54], CramoLL (*Cratylia molis*) [55,56], and BmoLL (*Bauhinia monandra*) [55] have already been used as biorecognition elements of arboviruses. Simão et al. [53] developed a nanostructured impedimetric biosensor to distinguish infections caused by different arboviruses - Dengue type 2, Zika, Chikungunya, and Yellow fever. The platform was built with ConA immobilized on zinc oxide nanoparticles/cysteine-modified gold electrode, which interact with mannose-glucose residues of the viral capsid. The bioaffinity of the biosensor for each virus changed (Zika > Dengue > Chikungunya > Yellow fever), justifying the differences for the attained LODs (0.0382 - 0.062 pfu mL^{-1}). There are also studies with Influenza A, i.e., a negative stranded RNA virus from the *Orthomyxoviridae* family that contains two useful surface glycoproteins (hemagglutinin and neuraminidase) used to classify viral subtypes. Anik et al. [57] investigated the potential of PNA lectin (*Arachis hypogaea*) in association with Fetuin-A glycoprotein/graphene–gold hybrid nanocomposite/gold-screen printed electrode as biosensing platform for Influenza H9N2 virus. Fetuin A includes terminal 12–14 sialic acid residues and, in the presence of neuraminidase, said glycoprotein is cleaved from these terminations [57,58]. The cleavage exposes galactose moieties that can be electrochemically screened by PNA with high specificity and sensitivity (LOD = 1.0×10^{-8} U mL^{-1}) in biological fluids. Additionally, Hushegyi et al. [59] showed that the *Maakia amurensis* lectin is also very useful to detect the influenza virus (by affinity for α-2,3-sialylated glycans), assessing neuraminidase enzyme activity. Very recently, it has been speculated that *Lablab purpureus* lectin has anti-influenza (H1N1) and anti-SARS-CoV-2 activity, becoming a promising macromolecule (not yet explored) for electrochemical biosensors designed to diagnose the related diseases [60]. The hemagglutinins of these viruses present a highly glycosylated protein called Env, which participates in mechanisms of infection and evasion of the host's immune system, promoting the fusion of the viral envelope to the plasma membrane of the infected cell. Lectins prevent this connection when they are previously bound to the aforementioned viral proteins. HIV-inhibiting activity was also observed with lectins from *Longisporum albid*, *Microcystis aeruginosa*, *Microcystis viridis*, among others, showing potential for employment in biosensing devices for the identification of the selected virus [61].

Escherichia coli (*E. coli*) is an enterohemorrhagic toxin-producing pathogen, being the predominant serotype associated with human health *E. coli* O157:H7. It causes infection via consumption of undercooked meat, unpasteurized milk, contaminated vegetables, and waters, among others. There are already reliable analytical methods for quality control of this pathogen in food, but they are usually time-consuming and require complex operational steps [62]. Alternatively, lectin-based electrochemical biosensors have been proposed to screen *E. coli*, especially configured with ConA lectin [63–68]. Yang et al. [67] used ConA immobilized on 11-mercapto-1-undecanoic acid self-assembled gold electrode to develop a label-free impedimetric biosensor for rapid detection of the non-pathogenic strain of *E. coli* DH5α, following the steps depicted in Fig. 4. The detection principle is based on the lipopolysaccharide existing on the surface of this gram-negative bacteria, achieving a LOD = 75 cell mL^{-1}. Li et al. [62] demonstrated that the wheat germ lectin (WGA) is also sensitive to *E. coli* O157:H7 (LOD = 10^2 CFU mL^{-1}) when this protein is immobilized on 3-dithiobis-(sulfosuccinimidyl-propionate) self-assembled screen-printed interdigitated gold electrodes. Other previously tested lectins for the same purpose were CramoLL [69], RCA [63], PNA, MAA, and those from *Triticum vulgaris* and *Ulex europaeus* [64] (not included in Table 1 since these studies were published > 6 years ago).

Fungi are also among the pathogens with bioaffinity for lectins. Special attention is given to those belonging to the *Candida* genus (*C. albicans, C. krusei, C. parapsilosis, C. tropicalis, C. glabrata*, among others opportunistic microorganisms), which cause candidiasis and candidemia in nearly 300 million people worldwide [70]. Therefore, their early detection helps in the proper prognosis and antifungal treatment, besides reducing costs, clinical care time and other hospital demands. Since *Candida* cell wall is composed of about 90% carbohydrates, lectins can be used for electrochemical biosensing. Sá et al. [70] proposed impedimetric biosensors for *Candida* spp. (*C. albicans, C. krusei*, *C. tropicalis*, and *C. parapsilosis*), using ConA and WGA as recognition agents of the yeast cells. Lectins were adsorbed on 4-mercaptobenzoic acid and gold nanoparticles-based composite/cysteine/gold electrode, allowing to differentiate species within the range of 10^2 to 10^6 CFU mL^{-1}. Speed and reproducibility of the measurements were the main advantages of these biosensors when compared with the traditional methods.

2.4. Cancer biomarkers

The discovery of drugs that prevent the uncontrolled multiplication of cancer cells from their initial stages remains a major challenge, so there is a great need for effective devices and methods for early diagnosis. Cancer cells have well-developed carbohydrate moieties on their surface, which are responsible for defense, recognition and cell-cell interaction mechanisms. The glycocalyx can also participate in metastasis processes [71]. In other words, glycan expressions transfer information about the physiological stage of various carcinomas, which are crucial for early diagnosis and treatment of the disease. In Table 1 (fourth section), it is possible to identify several lectins used for electrochemical biosensing of these biomarkers.

Some studies have associated overexpression of sialic acid derivatives with the development and progression of breast, prostate, colorectal and hepatocellular cancers. SNA and *Maackia amaurensis* (MAL) lectins can

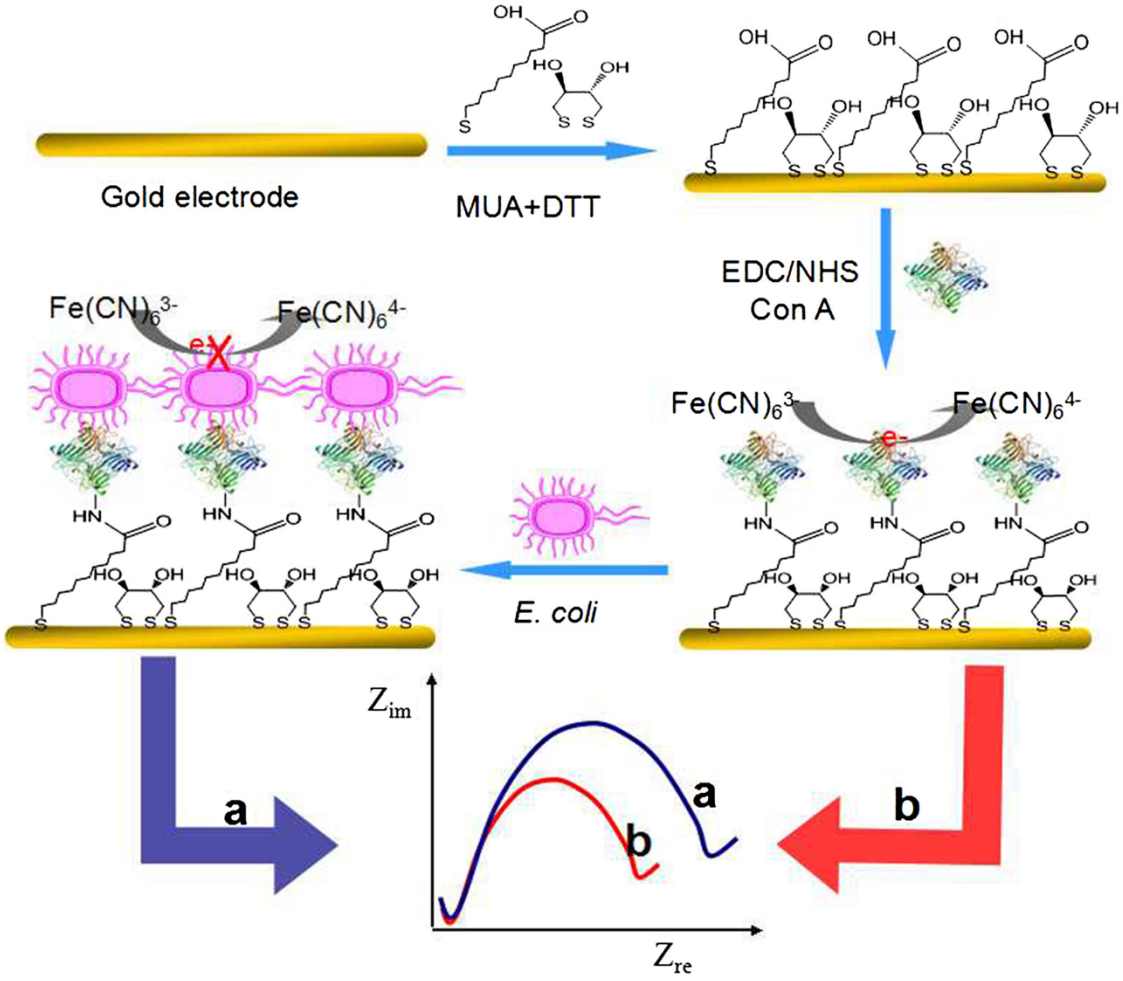

Fig. 4. Stages of the construction of a ConA-based impedimetric biosensor used for determination of *E. coli* DH5α. Adapted from Yang et al. [67] with permission from Elsevier.

recognize α-2,6-sialylated and α-2,3-sialylated glycans, respectively, justifying their uses in electrochemical biosensors for the carbohydrates mentioned [72–79]. Niu et al. [76] developed a highly-sensitive electrochemical device to detect α-2,3-sialylated glycans (LOD = 3.0 fg mL^{-1}) in human serum by EIS, using a GCE modified with carboxylated multi-walled carbon nanotubes (COOH-MWCNT) and polyamidoamine dendrimers (PAMAM). The authors used *p*-phenylene diisothiocyanate for immobilizing MAL on PAMAM@COOH-MWCNT/GCE, and the resulting biosensor was used to quantify the target carbohydrate with high accuracy. Another proposal in the same context was reported by Li et al. [78], which produced a sandwich biosensor for detecting α-2,6-sialylated glycans in human serum, using a GCE modified with streptavidin-associated gold nanorods and biotinylated SNA. The combination of these materials allowed a biocompatible microenvironment, improved lectin adhesion and immunoreaction efficiency. This label-free biosensor achieved a LOD = 0.50 ng mL^{-1} by using DPV, but the authors realized that the sensitivity could be even better (LOD = 0.69 fg mL^{-1}) when a carboxylated single-walled carbon nanohorns/sulfur-doped platinum nanocluster was adopted as signal label, producing a sandwich-type biosensor that exhibited excellent recovery and stability through chronoamperometric analysis.

SNA and MAL are also widely used to identify prostate-specific antigen (PSA), i.e., a biomarker for prostate cancer (PCa) diagnosis and screening [80–83]. Pihíková et al. [80] developed label-free EIS methods for detecting and glycoprofiling PSA (LOD < 4 amol L^{-1}). Direct detection was performed with a biosensor based on mixed SAM (11-mercaptoundecanoic acid and 6-mercapto-1-hexanol)/gold electrode to anchor SNA and MAL lectins. Regarding glycoprofiling, a sandwich immunosensor was structured with a monoclonal IgG antibody (anti-PSA) covalently attached on the SAM-modified gold electrode, which was subsequently incubated with SNA lectin to screen the disease-specific glycoprotein. Bertok et al. [83] conducted a proof-of-concept study to assess possible improvements for diagnosing PCa in the future, exploiting gold nanoshells with a magnetic core (Mn-Zn ferrite@silica@Au) to adsorb anti-PSA antibody (Fig. 5). This nanocomposite associates surface plasmons and magnetic attraction of core and shell layers, respectively, for spontaneous immobilization of zwitterionic molecules via diazonium salt grafting. After appropriate incubation time, anti-PSA antibody/Mn-Zn ferrite@silica@AuNPs structures were biorecognized by a SNA/carboxybetaine-bearing SAM-modified gold electrode in fortified human serum (LOD = 1.2 fmol L^{-1}), using EIS as electroanalytical technique. The developed methods are highly useful for clinical diagnostics considering that physiological PSA levels in human blood are below 130 pmol L^{-1} [80].

Several ConA-based cytosensors have been used for mannose biorecognition, since this carbohydrate is commonly present on animal cell surfaces [71,84–89]. Liu et al. [84] showed that ConA is useful for anchoring antibodies in a three-dimensional electrochemical immunosensor engineered for the detection of carcinoembryonic antigen (CEA), i.e., a biomarker overexpressed in pancreatic, lung, colon, breast, ovarian, and cervical tumors. Monolithic and macroporous graphene foam served as scaffold for the lectin immobilization, after its functionalization with polydopamine. The ConA-sugar-protein interaction was evaluated through immunoassays performed with horseradish peroxidase (HRP)-labeled antibody (anti-CEA), which demonstrated an

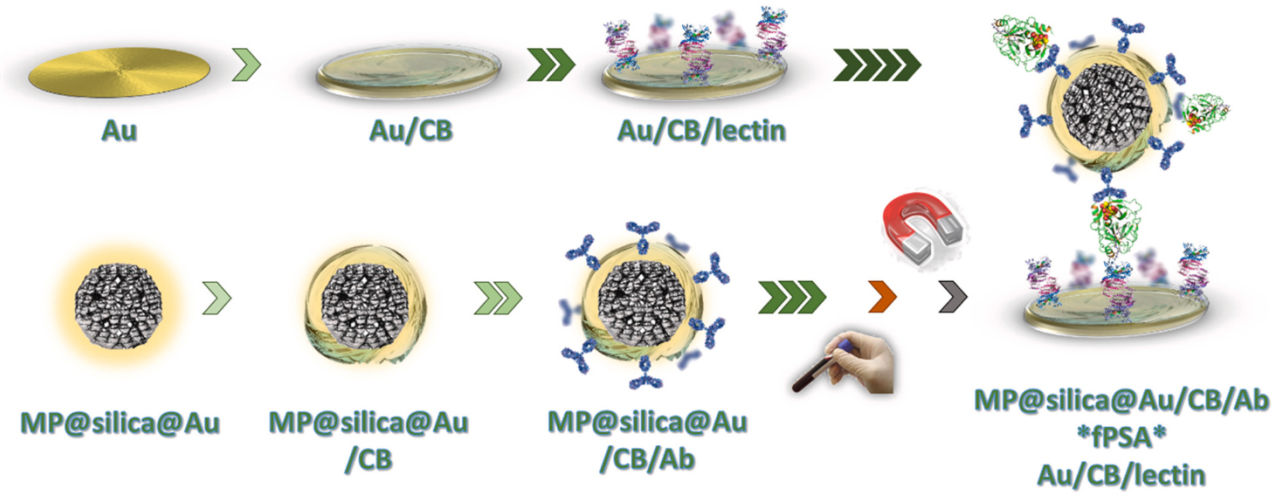

Fig. 5. Construction of SNA-I/carboxybetaine-bearing SAM/gold electrode and anti-PSA antibody-modified magnetic core-shell used in the biosensing detection scheme.
Adapted from Bertok et al. [83] with permission from Elsevier.

efficient biorecognition with LOD ~ 90 pg mL^{-1}, using DPV. Zhang et al. [88] innovated the cytosensing of the human breast cancer cell MCF-7, using a ConA/cysteine-terminated peptide self-assembled gold electrode and EIS (Fig. 6). Glycans over-expressed on the cell membranes were monitored for bioaffinity against alginate-loaded glucose oxidase, used as redox probe to improve the signal magnitude and detection capacity. The proposed electrochemical biosensor reached a LOD = 150 cells mL^{-1}, becoming a promising analytical tool to monitor the biomarker and diagnose the pathology early. Knowing that HRP and CEA bind to ConA through sugar-lectin interactions, Wang et al. [89] studied a label- and antibody-free electrochemical sandwich CEA biosensor, intercalating the mentioned antigen between lectin and DNA aptamer (5′-SH-$(CH_2)_6$-ATACCAGCTTATTCAATT-3′) layers. ConA also served as HRP anchor link that, in turn, was used for production and amplification of the electroanalytical signal (LOD = 3.4 ng mL^{-1}) recorded by DPV to quantify CEA in human serum.

Cancer cell biorecognition has been also performed with lectin such as WGA [86,87,90,91], *Cratylia mollis* (CramoLL) [92], *Lens culinaris* (LCA) [86,93], AIA [94], *Vicia villosa* (VVA) [95], *Lotus tetragonolobusa* (LTL) [96], *Phaseolus vulgaris* (PHA-E) [96] and *Arachis hypogaea* (PNA) [97]. Yang et al. [90] produced an electrogenerated chemiluminescence (ECL) biosensor for prostate PC-3 cancer cells, using anti-PSA as capture probe and ruthenium complex-labelled WGA as signal probe. This lectin has specific binding capacity to *N*-acetylglucosamine (GlcNAc) of *N*-glycans on the cell surface, and when it is labeled with the ruthenium complex, a high and regenerable ECL signal is observed, allowing to quantify very low levels of PC-3 (LOD = 2.6 $\times 10^2$ cells mL^{-1}). The authors also showed that the proposed ECL biosensor had an improved selectivity, when compared to the unlabeled WGA-based biosensor, to detect PC-3 in the presence of other cancer cells (Ramos and K562 cells). In addition, Zhao et al. [86] developed an electrochemical lectin-based biosensor array for sensitive detection and discrimination of CEA,

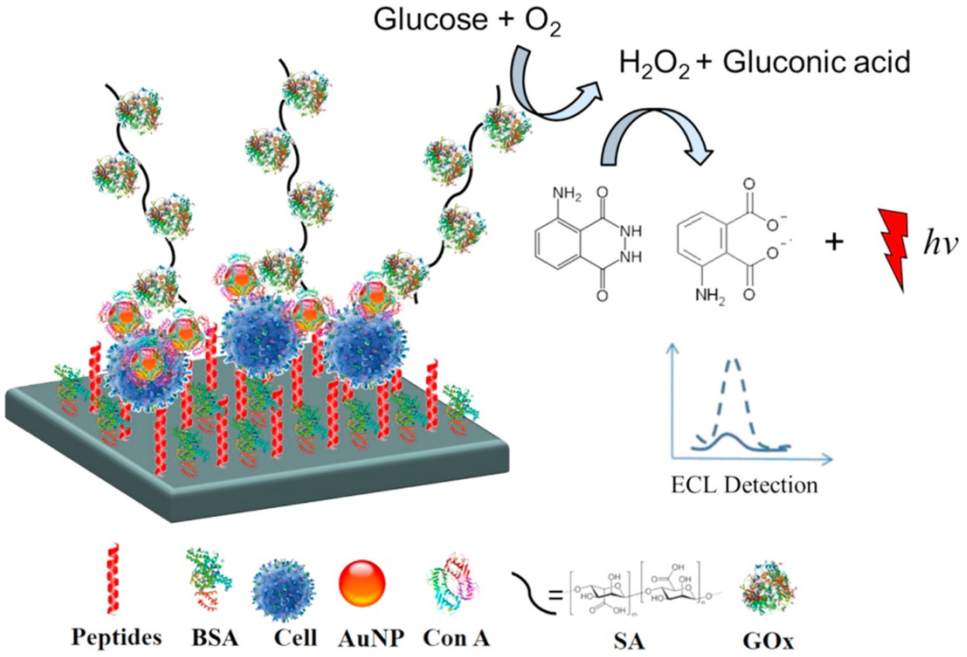

Fig. 6. Configuration of the electrochemiluminescent ConA-based biosensor and detection principle adopted for specific recognition of cancer cell MCF-7.
Adapted from Zhang et al. [88] with permission from Elsevier.

employing ConA, WGA, or LCA as molecular recognition elements. The selected lectins were immobilized on cysteamine self-assembled gold nanoparticle-modified screen-printed carbon electrodes, and lectin–target-antibody sandwich-type conjugates were formed when the biosensor interacted with a HRP-labeled anti-CEA antibody probe. The three designed biosensors reached low LOD values (0.01–0.05 ng mL^{-1}) and showed suitable performance to discriminate CEA *N*-glycan between healthy and cancer-stricken serum samples.

The enzyme β-1,4-galactosyltransferase plays an important role in the cellular processes and progression of lung cancer, and its electroanalysis intermediated by xanthine oxidase and AIA multi-labeled gold nanorod nanoprobes has been documented (Fig. 7) [94]. The resulting ECL biosensor operated in the presence of luminol, hypoxanthine and oxygen, and allowed to monitor AIA-galactose interactions as low as 9×10^{-4} U mL^{-1} enzyme [94]. Subtypes of α-fetoprotein (AFP), including LCA nonreactive, LCA-weakly reactive and LCA-reactive AFP (AFP-L3), are important for hepatocellular carcinoma (HCC) diagnosis, but the detection process remains unsatisfactory because the methods have low analytical sensitivity and require complex operational steps. Li et al. [93] proposed a simple and sensitive alternative to detect AFP-L3, using biotinylated LCA-integrated silver nanoparticles as recognition probe. In this proposal, electrochemical biosensing was performed with an AFP-L3/chitosan/graphene oxide/GCE platform, obtaining a LOD = 12 pg mL^{-1}, besides good stability and reproducibility for HCC clinical diagnostics. The presence of PCa and other tumor-associated antigens (Tn and T, for example) in biological samples were also studied with lectin-based biosensors built with VVA [95], PNA [97], and CramoLL [92], demonstrating the biotechnological relevance of these proteins for knowledge and progress in glycomics. Additional target glycoproteins with aberrant glycans are CA125 for ovarian cancer; hCG for testicular and ovarian cancer; CA15-3, CA27-29 and HER2 for breast cancer; and CA19-9 for pancreatic and ovarian cancer [98,99].

3. Challenges and perspectives

The regioselective complexation between lectins and carbohydrates or glycoconjugates explains the growing use of these systems to engineer electrochemical biosensors for clinical diagnosis in recent times. In contrast, lectin microarray models need further advances to meet the urgent demands for multiplexed analysis of sugar moieties in biological systems. When these proteins are organized in a microarray format, it is possible to perform a high-throughput carbohydrate profiling of structures with subtle structural differences [100]. Even so, it must not be forgotten that the sensitivity of these technologies is generally limited to sub-nanomolar levels, thus analyte labeling and quenching strategies need to be applied (through laborious and expensive procedures) in order to improve the performance [80].

Innovative platforms that combine sensitivity, specificity and label-free operation mode are intensively sought and, in these cases, lectin-based biosensors stand out. These devices have a remarkable ability to recognize lectin-carbohydrate interactions in real time, even considering a single analyte molecule [80]. Multiple functionalities are incorporated when associating lectins in biocomposites with semiconductors derived from carbon allotropes, metallic nanoparticles, polymers, coordination compounds, (in)organic-(in)organic hybrid structures, among others, which are arranged on sophisticated platforms of micro- and nano-metric dimensions. Selectivity and controlled orientation of proteins still demand more in-depth research, but such initiatives have already started from cutting-edge research involving functionalization of supporting materials, as well as recombinant protein techniques [101]. Certainly, these analytical tools will continue to provide important advances in clinical expertise with carbohydrates and glycoconjugates going forward.

4. Conclusions

Lectins and their carbohydrate-binding activities have inspired modern biotechnological solutions to problems faced in several fields. Lectin-based electrochemical biosensors deserve special attention for their remarkable ability to recognize relevant carbohydrates and glycoconjugates in complex samples, even without labeled analytes. They are also highly useful for profiling sugars moieties on biochemical targets, such as glycoproteins, pathogens and biomarkers. These platforms combine exceptional analytical sensitivity, specificity and precision, but their miniaturization is critical to address highly desirable features for point-of-care measurements, including portability, better signal density,

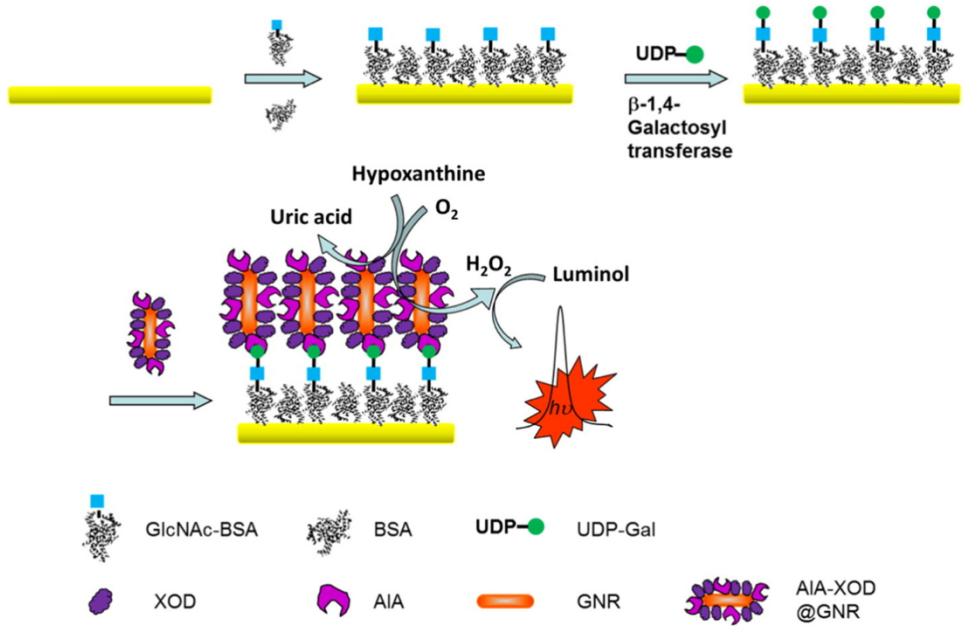

Fig. 7. Preparation process of an electrochemiluminescent biosensor for galactosyltransferase activity analysis, integrating a dual signal amplification strategy from the xanthine oxidase and AIA multi-labeled gold nanorod nanoprobes.
Adapted from Chen et al. [94] with permission from Elsevier.

fast response time, low-cost operation and ease-of-use. Such properties improve even more when the biosensors are assembled with biocompatible micro- and nano-structured semiconductors, although the lectin microarrays achieve more promising results for multiplexed analysis. Whether with one or more bio-affinity platforms, electrochemical devices based on lectins have attracted researchers around the world with the ultimate goal of understanding and monitoring physiological and pathological events involving carbohydrates and glycoconjugates. Since electrochemical biosensors enable the study of lectin-carbohydrate interaction transients at the molecular level, unimaginable advances driven by these technologies are expected in glycomics and related areas.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of interest

The authors declare no conflict of interest.

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