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## **Biomimetic vesicles for electrochemical sensing**

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### **Abstract**

Biomimetic vesicles, mainly composed of self-assembled bilayers of phospholipids, have attracted great attention for applications in the biosensor field over a number of decades, as a means to amplify the signal through encapsulated signal probes. In this review paper the most important developments in biomimetic vesicles for electrochemical biosensing within the last 2 years are presented, with a focus on the format of bioassays, their inclusion in microfluidic chip devices and their use in mimicking cell membranes. Key issues and the remaining challenges for future commercialization are analyzed.

### **Introduction**

Synthetic or natural surfactants that can self-assemble as bilayers are the elementary molecules of vesicles or liposomes. The most common surfactants forming liposomes are phospholipids, the surface-active compound present in cell membranes; liposomes can then mimic biological membranes. The structure of vesicles depends on the dispersion process [1]. The most common structures are multilamellar large vesicles (MLV), small unilamellar vesicles (SUV) of sub-micron diameter made of a single closed bilayer membrane, and giant unilamellar vesicles (GUV) of a few tens of microns in diameter.

Water-soluble agents can be encapsulated in the inner cavity of the vesicle; water-insoluble agents can be incorporated into the bilayer membrane. Membrane permeability can be greatly reduced by the addition of cholesterol to the bilayer membrane of phospholipids [2]. In addition, phospholipids, as the main component of biomimetic vesicles, have distinct advantages over synthetic materials including lack of toxicity, biodegradability and biocompatibility.

Consequently, biomimetic vesicles are utilized as versatile carriers in medical, therapeutic, and analytical applications.

Biomimetic vesicles have attracted great attention for applications in the biosensor field over a number of decades as a means to amplify the signal [3,4]. Biomimetic vesicles can encapsulate various signal probes including dyes, enzymes, salts, chelates, electrochemical and chemiluminescent probes. Consequently, biomimetic vesicles are an excellent candidate component for biosensors to transduce and amplify signals.

Biomimetic vesicles can also serve as cell membrane models because, like liposomes, they have similar components and structure to cell membranes. They can therefore provide a simple platform to simplify the investigated system in the research into related interactions and physiological phenomena with cells [5].

The technology for the surface modification of vesicles ensures that a variety of biorecognition elements can be conjugated to the surface of liposomes, including peptide, protein, enzyme, antigen, biotin, avidin and DNA segments. For lysis strategy, surfactant (or detergent) and natural lytic agents, such as pore-forming toxins [6], have been reported.

This review paper focuses on biomimetic vesicles for electrochemical biosensing. Electrochemistry is a sensitive, fast and convenient analytical technique widely used in the sensor field. There are several advantages to electrochemical detection. First, the electrochemical signal is a stable and sensitive signal that can be rapidly and easily detected. Secondly, the electrochemical devices are readily miniaturized for the development of portable sensors, without the need for larger detectors. Therefore, electrochemical biosensors based on electrochemical probes encapsulated in biomimetic vesicles as a signal amplifier, have attracted great attention in biochemical analysis.

### **Biomimetic vesicles in electrochemical bioassays**

The general schematic for how a liposome might be deployed in a simple biosensor is illustrated in Fig. 1. A number of different types of vesicle-based assays have been reported using biomimetic vesicles as a signal amplifier, including vesicle immunosorbent assay (VISA) (Fig. 1A) and vesicle DNA hybridization assay (V-DNA-HA) (Fig. 1B), electrochemical redox probes encapsulated in biomimetic vesicles as signal amplifiers being successfully utilized in these assays.

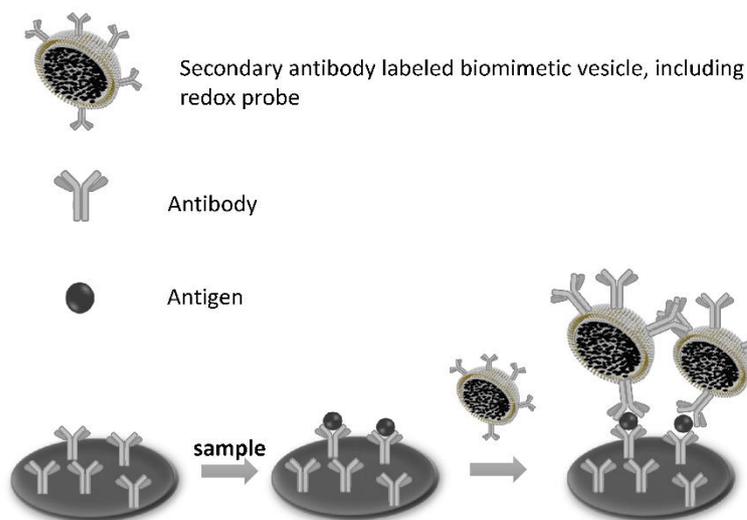


Figure 1A : Vesicle immunosorbent assay (VISA). Vesicles are presented as hemivesicles to show the inside.

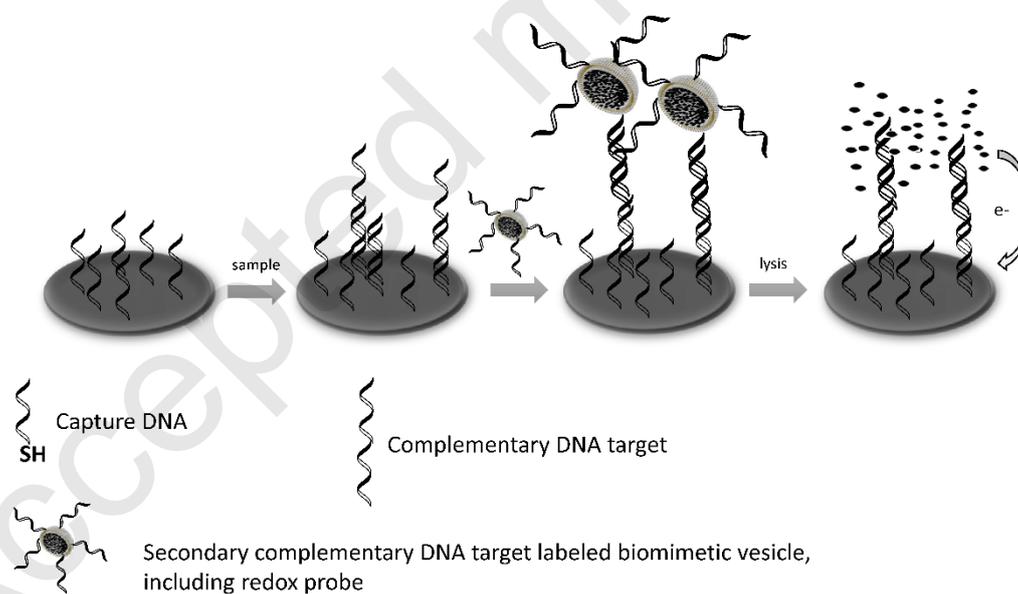


Figure 1B : Vesicle DNA hybridization assay (V-DNA-HA). Vesicles are presented as hemivesicles to show the inside.

Electrochemical VISA has been used for the detection of carcinoembryonic antigen, cholera toxin and insulin based on a combination of traditional VISAs and the electrochemical

technique. Ascorbic acid, uric acid, ferrocene carboxylic acid, potassium ferrocyanide and ferrocene were used as the electrochemical probes for the detection of carcinoembryonic antigen at  $5 \times 10^{-7}$  g/mL [7], cholera toxin at  $1 \times 10^{-16}$  g [8] and insulin at 10 pg/mL [9], respectively.

Nucleic acid sequences and *Escherichia coli* O157 based on V-DNA-HA [10, 11] were detected using hexaammineruthenium(III) chloride-encapsulated vesicle electrochemical probes. Based on the same principle, a PCR-free and highly sensitive detection of human telomerase activity was reported. Using dopamine-loaded biomimetic vesicles, the telomerase activity extracted from 10 cultured cancer cells could be detected [12•].

Vesicle-gold nanoparticles were stably grafted on a thiol monolayer (short alkane chain) modified gold electrode surface [13]. A low detection limit of DNA ( $10^{-14}$  M) was obtained through electrochemical impedance spectroscopy in the presence of potassium ferrocyanide [14]. A lower detection limit of DNA ( $10^{-15}$  M) was obtained when vesicle-gold nanoparticles were physisorbed on thin layered rGO [15].

Secondary electrochemical signal amplification was also reported by Chu et al. to detect human prostate specific antigen (PSA) through encapsulation of alkaline phosphatase (ALP) in vesicles and relying on a sandwich VISA [16]. In this strategy, ALP was utilized as the signal marker for electrochemical signal amplification, using its substrate ascorbic acid 2- phosphate (AA-p). A detection limit as low as 0.007 ng/mL for PSA was detected using this approach. ALP loaded vesicles have also been used for HIV-p24 antigen detection. The produced ascorbic acid donated an electron to the graphene/g-C<sub>3</sub>N<sub>4</sub> nano hybrid based photoelectrode, provoking an increased photocurrent signal. A detection limit of 0.63 pg/mL was obtained with the proposed PEC method [17].

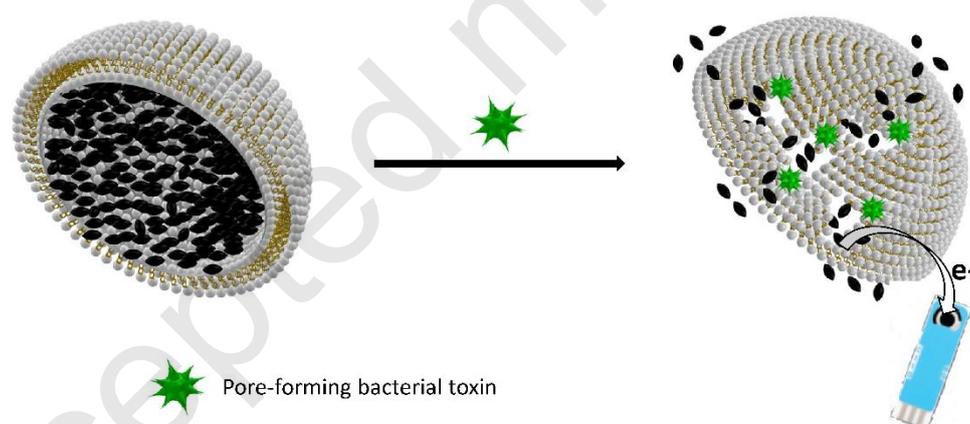
### **Biomimetic vesicles in electrochemical microfluidic chip devices**

In the 90s, a series of vesicle-based immunoassays with a strip format, working on a lateral flow principle, were developed; the first one was proposed by Durst [18] for the detection of the herbicide alachlor. Recently, microfluidic chips produced by means of microtechnology facilities, were preferred. The advantages of the vesicle-based microfluidic chip are the shortening of detection time to only 20 min, and a significantly lower limit of detection, down to pmol/mL. For example, a low concentration of Dengue fever virus was detected using the vesicle-based microfluidic chip through a sandwich DNA hybridization assay [19,20]. Cholera toxin was detected in fecal samples by Baeummer et al using a microfluidic biosensor; the toxin

was captured through anti-CTB (cholera toxin subunit B) antibody conjugated magnetic beads, a GM1-containing vesicle being then linked to CTB; the magnetic immobilization of the magnetic bead, the washing step, the vesicle lysing and the ferri/ferrocyanide detection were performed in the microfluidic chip device. A detection limit of 31.7 ng/mL was obtained [21].

### Mimicking cell membranes for biosensing

Synthetic vesicles or liposomes based on phospholipids mixed with polyacetylene have been extensively used for mimicking cell membranes [5]. For this purpose, the molecular system produced should retain, as much as possible, the physico-chemical properties of the actual cell membrane (such as lipid and protein organization and fluidity). The elaboration of biosensors for hemolytic bacteria is based on the detection of their emitted toxin that has the specific property of forming pores in the cell membrane. The redox-encapsulated vesicle is lysed under the influence of the species presenting pore-forming functions such as bacterial toxins (Figure 2).



**Figure 2 :** Amperometric biosensing of pore-forming bacterial toxin based on biomimetic vesicle encapsulation of redox probes. Vesicles are presented as hemivesicles to show the inside.

Pathogenic bacteria produce a large variety of toxins and virulence factors. Hemolytic bacteria are pathogenic bacteria that produce pore-forming toxins, ultimately resulting in cell death by necrosis or apoptosis [22]. Biomimetic vesicles have been synthesized to detect this type of toxin through electrochemical methods. To mimic the cell membrane, the mixed bilayer is composed of a mixture of phosphocholine, mixed with diacetylene monomers and cholesterol

as a bait molecule, since the first step for pore formation is believed to be the toxin binding to cholesterol. For electrochemical detection, redox compounds such as ferrocene, hexacyanoferrate or 2,6-dichlorophenolindophenol, are entrapped in the vesicles [23-26] or inserted in the bilayer membrane [23,25]. Detection limits of bacterial toxins were 0.025 nM for streptolysin O [23], 36 nM for *E coli* heat-labile enterotoxin [24], 11  $\mu$ M for rhamnolipid and 20  $\mu$ M for delta toxin [26].

The use of non-Faradaic liposome rupture impact voltammetry was able to qualitatively detect a model amphiphatic viral peptide on a screen-printed electrode. AH peptide was detected at the level of 26  $\mu$ M [27<sup>••</sup>] through the formation of a bilayer on the electrode surface after rupturing.

Lipid phosphorylation plays a central regulatory role in various fundamental cellular processes. Sphingosine-containing vesicles were phosphorylated through an enzymatic reaction with kinase and then coordinated on a NTA-Fe(II) modified gold electrode. The SWV (square wave voltammetry) signal of released methylene blue is a function of kinase activity. A detection limit of 2.33 pmol/min/mg was obtained [28].

Biomimetic vesicles encapsulating potassium hexacyanoferrate have been immobilized on a SAM modified gold electrode, allowing for the first time the detection of a conformational change in proteins (bovine carbonic anhydrase and lysozyme). A linear relation between the output amperometric signal and denatured concentrations was obtained [29].

### **Biomimetic vesicles for biosensing towards potential commercialization**

In this final section, progress towards the commercialization of a vesicle-based diagnostic chip and related techniques are discussed.

A new technology based on a commercial personal glucometer has been developed to quantitatively detect a broad range of disease biomarkers and was proven to be portable, economical and conveniently accessible. Measurements were performed based on releasing encapsulated glucose from antibody-tagged vesicles and subsequently detecting the released glucose using a commercial glucometer. The innovative aspect of this approach lies in the quantification of target biomarkers through the detection of glucose, thus expanding the applicability of the glucometer by broadening the range of target biomarkers instead of detecting only one analyte, glucose. Because of the bilayer membrane of biomimetic vesicles, which can accommodate tens of thousands of glucose molecules, the sensitivity was greatly enhanced by using glucose-encapsulating vesicles as signal output and amplifier. Based on this

original concept, biomarker phospho-p53 has been detected, with a detection limit of 50 pg/mL [30] and aflatoxin B1, a contaminant of foodstuffs, has been detected with a detection limit of 0.6 pg/mL [31]. Thrombin was also detected using a commercial glucometer: 29-mer aptamer against thrombin functionalized glucoamylase encapsulated vesicles were used, allowing a secondary enzymatic amplification, in the presence of the enzymatic substrate amylose [32]. Following the same design, DNA methyltransferase activity was detected [33<sup>••</sup>].

Several biomimetic vesicle-based assays were conducted in real samples: AFB1 was detected in contaminated/spiked peanuts samples and serum samples, using glucometer [31; the practicability of the liposomes-amplified PEC sensing strategy was demonstrated by assaying human serum samples and its universality was also demonstrated by developing it into a sensitive microRNA detection method [17].

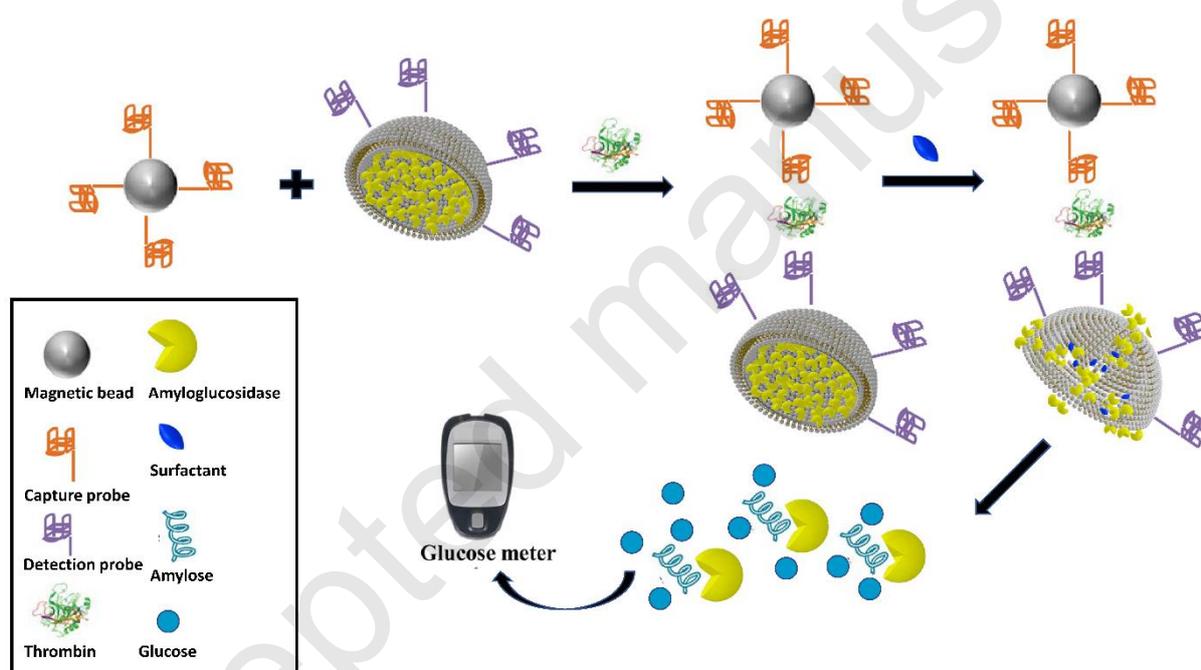


Figure 3 : Working Principle of Enzyme-Encapsulated Liposome-Linked Immunosorbent Assay with Beads/Protein/Liposome “Sandwich” Structure [32].

As a potential tool for point-of-care diagnosis, vesicle-based microfluidic chips based on the combination of biochip technique and vesicle-based signal amplification have good commercial prospects in point-of-care diagnosis. One of them was used for the detection of cholera toxin detection in fecal samples [21<sup>•</sup>]. However, several problems still limit the development of commercialized vesicle diagnostic products, such as leakage of probe molecules and poor stability. Solutions to these problems and the development of suitable and robust vesicle

systems for commercial applications are necessary for the commercialization of vesicles in the biosensing field.

Stability is a critical feature in the commercialization of vesicle-based biosensors because commercial vesicle-based diagnostic reagent kits or devices are normally required to be stored longer than 1 year. The incorporation of some molecules into lipid bilayers is helpful to enhance their stability: cholesterol, because it weakens the interactions between the acyl chains of phospholipids, sugars such as trehalose that protect vesicles during freezing and freeze-drying, cross-linkable polymers such as polydiacetylene or polyacrylic acid that strengthen the bilayer. It has been reported that with such a formulation biomimetic vesicles could be freeze-dried and stored for 3 months, their structure and the encapsulated calcein being preserved [34].

Several strategies have been developed to avoid the fusion of vesicles in suspension, because unilamellar vesicles tend to fuse into large vesicles in suspension, such as the adsorption of carboxyl-modified polystyrene nanoparticles [35], or coating with diethylaminoethyl dextran [36].

Redox-inactive molecules-encapsulated vesicles could also be used in electrochemical bioassays. It has been reported that electrochemical nanoimpact titration could allow the determination of the attomole content of redox-inactive molecules such as glutathione within individual vesicles, by using copper (II) as a catalyst [37].

A continuous process to produce hybrid liposome/protein microvesicles has been reported using microfluidics and electrospray [38<sup>••</sup>], opening the way to an industrial process for producing biomimetic vesicles.

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Telomerase, which has been detected in almost all kinds of cancer tissues, is considered as an important tumor marker for early cancer diagnostics. In the present study, an electrochemical method based on liposomal signal amplification platform is proposed for simple, PCR-free, and highly sensitive detection of human telomerase activity, extracted from A549 cells. In this strategy, telomerase reaction products, which immobilized on streptavidin-coated microplate, hybridized with biotinylated capture probes. Then, dopamine-loaded biotinylated liposomes are attached through streptavidin to biotinylated capture probes. Finally, liposomes are ruptured by

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This biosensor was previously developed and tested in buffer solutions only, using either fluorescence or electrochemical detection strategies. The microfluidic devices were made from polydimethylsiloxane using soft lithography and silicon templates. Cholera toxin subunit B (CTB)-specific antibodies immobilized onto superparamagnetic beads and ganglioside GM1-containing liposomes were used for CTB recognition in the detection system. Quantification of CTB was tested by spiking it in human stool samples. Subsequently, cross-reactivity using the heat-labile *Escherichia coli* toxin was investigated using the electrochemical microfluidic immunosensors and was determined to be negligible.
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- Detection of infectious viruses and disease biomarkers is of utmost importance in clinical screening for effective identification and treatment of diseases. We demonstrate here the use of liposome rupture impact voltammetry for the qualitative detection of model amphipathic viral peptide on a screen-printed electrode. This novel, proof-of-concept method was proposed for the quick and reliable detection of viruses by nonfaradaic liposome rupture impact voltammetry with the aid of 1,2-dioleoyl-sn-glycero-3-phosphocholine liposomes.
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Microfluidics and electrospraying, two revolutionary technologies with industrial potential for the microencapsulation of lipophilic bioactive ingredients, have been combined to produce hybrid liposome/protein microencapsulation structures in a semi-continuous process, reducing the number of steps required for their manufacture. The Tesla design showed the best mixing performance, as observed by fluorescence microscopy, so it was selected to be assembled to an electrospraying apparatus. The proposed in-line setup was successfully used to produce the micron-sized encapsulation structures, as observed by scanning electron microscopy.