Review

Responsive polydiacetylene vesicles for biosensing microorganisms

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Abstract: Polydiacetylene (PDA) inserted in films or in vesicles have received increasing attention due to PDA property to undergo a blue-to-red colorimetric transition along with a change from non-fluorescent to fluorescent upon application of various stimuli. In this review paper, the principle of the detection of various microorganisms (bacteria: directly detected or detected through the emitted toxins or through their DNA, and viruses) and of antibacterial and antiviral peptides based on these responsive PDA vesicles are detailed. The obtained analytical performances, when vesicles are in suspension or immobilized, are given and compared to those of the responsive vesicles mainly based on the vesicle encapsulation method. Many future challenges are then discussed.

Keywords: vesicles; polydiacetylene; biosensing; bacteria; toxins; virus; peptides

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1. Introduction

The demand for new sensing technologies that can serve as alerts for bacterial contamination has significantly increased in recent years because of incidents of food poisoning, bioterrorism alerts, and anthrax scares. Numerous technologies for bacterial detection have been developed [1]. Nevertheless, many methods employed for pathogen sensing provide results after relatively long time spans (several hours to days in the case of culture based methods). Other currently employed technologies often involve complex detection mechanisms that require specialized instrumentation, trained personnel, and the need for complex sample preparation, which overall do not make possible uses in settings elsewhere than laboratory environments.

Polydiacetylene (PDA) has attracted significant scientific and technological interest in recent years because of its unique chromatic properties. Specifically, PDA was shown to self-assemble into organized vesicles and films, forming an ene-yne conjugated framework that absorbs light in the visible region of the electromagnetic spectrum and consequently appears intensely blue [2]. Furthermore, it was shown that external perturbations, primarily affecting the reorganization of the pendent polymer side-chains as a result of enhanced surface pressure, give rise to stress induced structural transformations of the PDA backbone, resulting in dramatic blue-red transitions. PDA also exhibits interesting fluorescence properties; no fluorescence is emitted by the initially polymerized blue-phase PDA, whereas the red-phase PDA strongly fluoresces.

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Synthetic vesicles or liposomes based on phospholipids mixed with polyacetylene have been extensively used for mimicking cell membrane [3]. For this purpose, the molecular system produced should retain, as much as possible, the physical chemical properties of the actual cell membrane (such as lipid and protein organization and fluidity). The elaboration of biosensors for haemolytic bacteria is based on the detection of their emitted toxin that has the specific property of forming pores in cell membrane. Screening of molecules with antibiotic properties is also based on the specific properties of these molecules to from pores in cell membrane. This review paper reports the main recent papers that present PDA vesicle-based assays, involving this phenomenon, for the detection of bacteria, bacterial toxins and antibiotic peptides. The direct detection of bacteria based on the specific interaction with antibody and aptamer functionalized PDA vesicles is also reported and both principles of detection are compared in terms of selectivity and sensitivity. The direct detection of viruses based on the specific interaction with receptor functionalized PDA vesicles is also reported. The analytical performance of PDA vesicle-based assays are moreover compared to those of other types of responsive vesicles, involving mainly the vesicle encapsulation method.

Many reviews on synthetic vesicles are mainly focussed on vesicle encapsulation method that enhance the sensitivity of sandwich immunoassays [4,5]. This aspect will not be included herein.

2. Physicochemical characteristics of PDA vesicles

Structure and synthesis of PDA vesicles

Synthetic or natural surfactants that are able to 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 mimick biological membranes [3]. The structure of vesicles depends on the dispersion process [6]. The most common structures are large multilamellar vesicles (LMV), small unilamellar vesicles (SUV) of sub-micron diameter made of a single closed bilayer membrane, and giant uniilamellar vesicles (GUV) of few ten micron diameter (Figure 1).

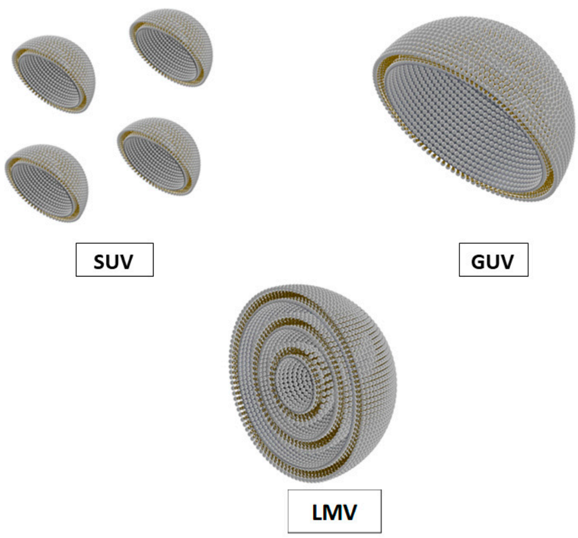

Figure 1: Different structures of vesicles: SUV (small unilamellar vesicles), GUV (giant unilamellar vesicles), LMV (large multilamellar vesicles).

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The PDA vesicles are all unilamellar vesicles composed of one spherical mixed bilayer encapsulating probes or not. The general procedure for their preparation is described as follows: A mixture of phospholipids and diacetylenic acid is dissolved in chloroform by vortexing and warming (at about 40 °C) until completely dissolved. [7]. The homogeneous mixture is then placed under vacuum until the complete evaporation of chloroform. The dry lipid film is hydrated by the addition of an aqueous solution and then the solution is mixed and heated on a hot plate at elevated temperature (80 °C) for around 30 min. The vesicle solutions are extruded at elevated temperature (80°C), several times, through polycarbonate membranes (400, 200 or 100 nm diameter) or sonicated. Polymerization of PDA is then performed under UV light (254 nm).
The study of the influence of the UV doses on the stability of vesicles has shown that a higher degree of PDA polymerization improves their overall stability [8]. Moreover, the passive leakage of entrapped probes (*i.e.* fluorescent probe) is minimized when the degree of PDA polymerization is increased [8]. The composition of the lipid mixture can influence the biomimetic behavior of the obtained film as demonstrated in Ref [9].

A size-controlled fabrication of supramolecular vesicles using a microfluidic chip was described [10]. The mean and standard deviation of the diameters of PDA vesicles produced by using the bulk method are respectively 88 and 31 nm and those of vesicles prepared with the microfluidic method are respectively 39 and 12 nm.

The colorimetric response of the PDA vesicles and formats of the PDA vesicle-based assays

One of the more fascinating aspects of polydiacetylene chemistry is the color and chromism of the materials. The energy of electronic excitations, and therefore the color of the material, can be dependent upon many factors such as the original packing state of the monomers and the exposure of the polymeric material to environmental perturbations such as heat (thermochromism), mechanical stress (mechanochromism) or solvent (solvatochromism). The blue to red transition is associated with a conformational change of the PDA backbone from planar to non planar and less conjugated, the side chains being more ordered in the red phase. The color transitions of the polymerized vesicles are monitored by visible absorption spectroscopy: 620-640 nm (PDA blue form) and 490-540 nm (PDA red form) [2]. PDA red form also presents a fluorescence emission in the range 520-700 nm, when excited at 488 nm. Fore example, colorimetric and fluorescent detection of melamine through PDA vesicles were compared. The intra/inter hydrogen bonding between melamine and cyanuric acid receptor at the PDA vesicle surface induces perturbation of the PDA backbone and results in rapid and sensitive colorimetric/fluorescence change of the PDA vesicle. A detection limit of 1 .0 ppm is obtained for colorimetric PDA liposome and 0.5 ppm for fluorescent PDA vesicle array [11].

The format of the colorimetric/fluorescence assay can be as a multiwell plate when vesicles are free in solution [see for instance Ref. 12]. In order to miniaturize the assay, patterned arrays are formed through the immobilization of vesicles on surfaces. Different surface modification were proposed for vesicle immobilization: aldehyde [13,14], amine [14,15] and α-cyclodextrin [16] funtionalizations. An interlinker, ethylenediamine, which acts as a cross-linker between individual PDA vesicles allows stabilization of PDA vesicles on solid surface and the fluorescence signal is ten times higher than for the array without the interlinker [17].

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The physico-chemical characterization of the PDA vesicles

Of the many physicochemical characterization methods used so far, light scattering and microscopy methods provide the clearest information regarding the morphology of vesicles.

Dynamic light scattering (DLS) measurements allow the diffusion coefficient of the vesicles in the liquid suspension to be determined. This diffusion coefficient is converted into a mean diameter by use of the Stokes-Einstein relationship under the hypothesis that vesicles are spherical in shape. This assumption can be unsuitable when vesicles are flexible and their shape strongly fluctuates; this is the cases of large unilamellar vesicles. DLS does not allow the user to infer the shape or discriminate between unilamellar and multilamellar vesicles, neither does it allow detection of pores or holes through the lipid bilayer, nor discrimination between closed bilayer (vesicles) and fragments of bilayers (nanodiscs). DLS provides quite satisfactory data in the case of vesicles smaller than 1 μm.

Transmission electron microscopy (TEM) provides high resolution pictures of the vesicles, allowing the discrimination of unilamellar and multilamellar vesicles, and possibly the thicknesses of the lipid bilayers and the water layers in between them (in case of multilamellar vesicles). Classical TEM requires the samples to be dried before observation, so drying is not expected to change the organization and the images should reveal the structure present in the aqueous suspension. It is often useful to enhance the contrast using heavy metal staining agents such as uranyl acetate. Again it is hoped that staining does not disturb the structure. Either cryo-TEM or TEM of a replica prepared by the freeze-fracture technique allow more reliable observations of the structure prevailing in the liquid suspension. An example of such images of small unilamellar and large multilamellar vesicles, made of bilayers of synthetic double chain zwitterionic surfactants [18], is given in Figure 2.

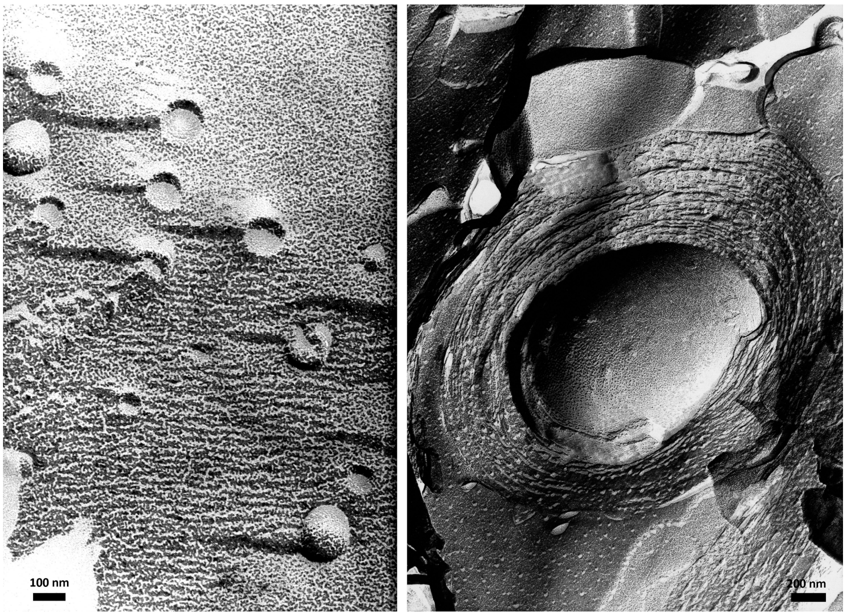

Figure 2. TEM images of small unilamellar vesicles (SUV, left) and large multilamellar vesicles (LMV, right), made of bilayers of synthetic double chain zwitterionic surfactants, taken after preparation by freeze-fracture and replication of the fracture section. SUV appear as small circles being either full or having an empty water pool inside depending on whether the fracture propagated across the vesicles or along their external surface. LMV appear as onion-like stacks of lipid bilayers. Such concentric bilayers fill the whole vesicle; the empty hole in the middle corresponds to part of the vesicle center that has been detached when fracturing the frozen sample (fracture took place in between the bilayers).

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The colloidal stability of PDA vesicles requires strong enough repulsions between them so as to prevent coagulation. Quite strong electrostatic repulsions come from the presence of the anionic carboxylic groups as heads of PDA chains. Electrostatic effects can be assessed by electrophoretic measurements of the zeta potential. Since most charged species are salts of weak acids, it is wise to measure the zeta potential as a function of pH and determine the isoelectric point. Efficient electrostatic stabilization requires that the pH is shifted by at least one or two units from the isoelectric point.

The most interesting formulations of lipid components on the organization of lipid membranes could also be investigated by Langmuir compression in a Langmuir trough. The pressure-area isotherms of mixed monolayers including the same lipid components are registered, giving information on the overall lipid compaction. The inverse of the two-dimensional compressibility of the monolayer as a function of pressure reflects the fluidity/elasticity of the monolayer [19]. The elasticity of a PDA mixed Langmuir film was studied in Ref [20].

Atomic force microscopy [21] and Total Internal Reflection Fluorescence (TIRF), allows the evaluation of the shape and of the viscosity of the individual biomimetic vesicles. TIRF single vesicle measurements were performed to determine the vesicle rupture lag time in the presence of antiviral peptides [22]. It was demonstrated that C5A peptide presents a potent vesicle rupture activity, this activity being independent of vesicle diameter. AH peptide is highly membrane-active while it preserves vesicle size-selectivity. This point influences the range of enveloped viruses that is targeted.

3. Transducing principles and preparation of responsive biomimetic vesicles

Two main transducing principles are implemented in responsive biomimetic vesicles:

1) Biomimetic PDA vesicles, PDA being used as a transducer for biological sensing, have been used as useful platforms for analysis and rapid screening of biomolecular recognition events [23]. Conjugated PDA is a remarkable polymeric system which exhibits unique organization and chromatic properties. This polymer has a strong blue color, due to electron delocalization within the conjugated double bonds, giving rise to an absorption at around 650 nm in the visible region of the electromagnetic spectrum. Importantly, PDA can undergo both rapid blue–red color transitions (upper lines in Fig. 3) and concomitant fluorescence transformations (lower lines in Fig. 3), induced by external stimuli such as surface binding, insertion or pore formation, which disturb electron conjugation of the polymer.

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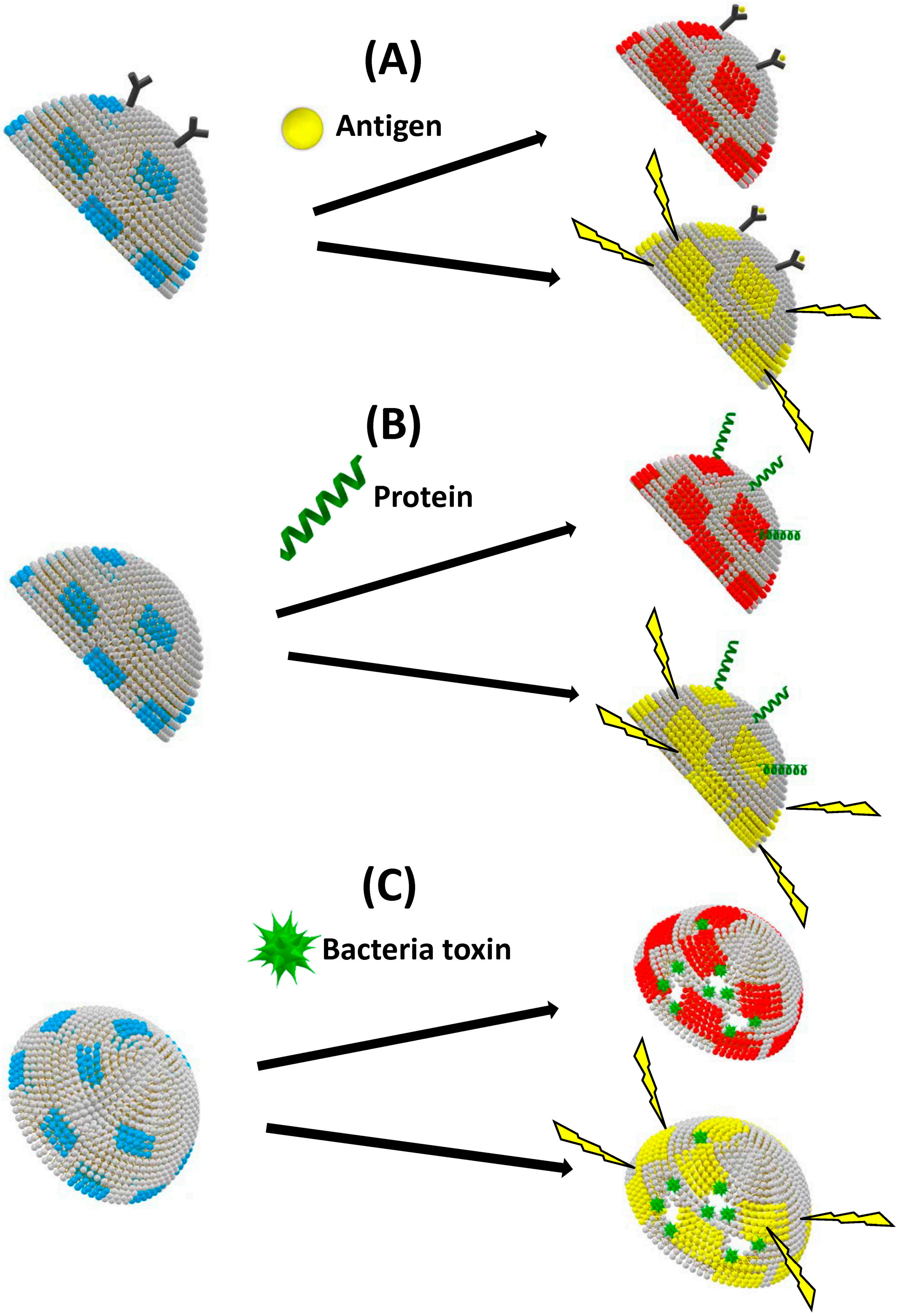

Figure 3: Colorimetric (upper line) and fluorescence (lower line) biosensing based on biomimetic vesicles comprising polydiacetylene (PDA) (blue and red parts) induced by external stimuli (A) Surface binding, (B) Insertion, (C) Pore formation

2) Other types of responsive vesicles are based on the vesicle encapsulation method: fluorescent dye or redox species being encapsulated in the vesicle. These assays were mainly for the

detection of species presenting pore-forming functions such as bacterial toxins or antibacterial substances (Fig. 4).

An example of fluorescent vesicle-based biosensor for organophosphorous pesticides (OP) detection was developed by encapsulating in an egg phosphatidylcholine liposome, the enzyme acetylcholine esterase and the pyranine fluorescent indicator. The enzyme substrate passes through porine channels and induces a decrease of pyranine fluorescence signal by decreasing the local pH. When enzyme is incubated with OP, the enzyme activity decreases, inducing an increase of the fluorescence signal in presence of the same concentration of substrate [24]. Another fluorescent liposome based system contains specific pyrenyl amphiphiles: the variation of excimer/monomer ratio upon the interaction with the target enzyme, thymidine phosphorylase, allows to specifically detect its presence [19].

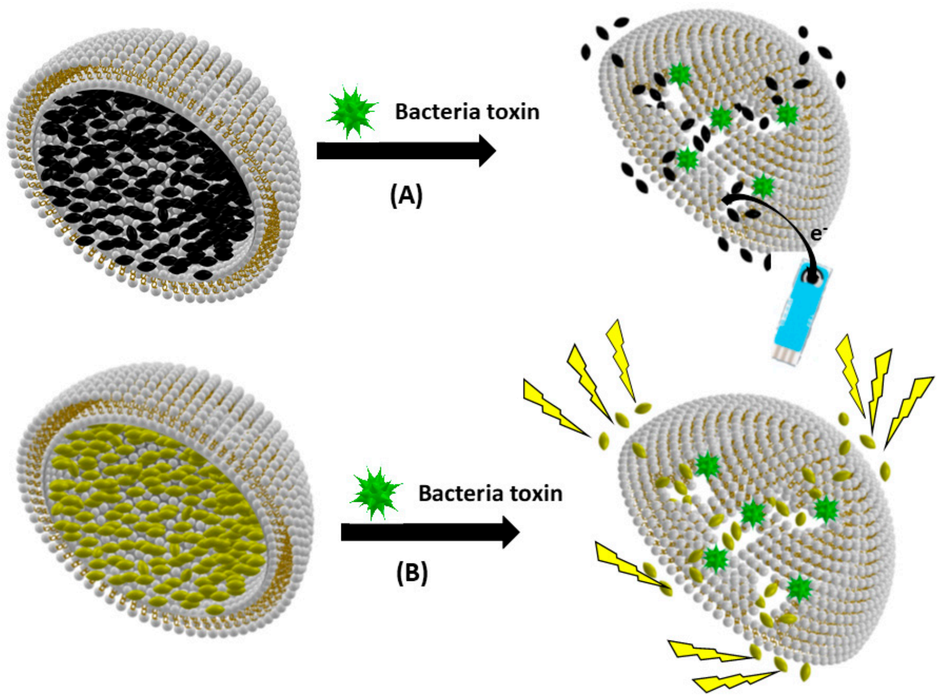

Figure 4: (A) Amperometric biosensing based on biomimetic vesicle encapsulation of redox probes (B) Fluorimetric biosensing based on biomimetic vesicle encapsulation of fluorescent probes

redox probe fluorescent probe

4. PDA vesicle-based assays for bacteria detection

4.1. Direct detection of bacteria

The published papers dealing with the direct detection of bacteria that use mixed PDA vesicles are based on optical detection: colorimetric detection due to blue-red transition of PDA under mechanical stress or fluorescence detection in the presence of a fluorophore grafted on a diacetylenic acid chain (Table 1). The interaction with the bacteria membrane can be ensured by the following specific molecules

1) either inserted in the bilayer membrane: long chain glucoside [25, 26, 28], sphingomyelin [31,32]
2) or grafted on the diacetylenic acid chain: antibody [28], aptamer [30]

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The colorimetric detection of ligand/receptor interactions through physical incorporation of receptors within lipid/PDA vesicles offers important advantages over chemical attachment of recognition units to the PDA itself. First, the chemical derivatization of PDA can be technically demanding and the organic synthesis procedures limit the scope of this approach. Furthermore, attaching additional chemical units onto the diacetylene monomers often disrupts the organization and the self-assembly of the monomers and hence compromises polymerization. Consequently, the abundance of recognition modules in derivatized PDA vesicles is low. Such limitations are generally not encountered when the recognition element is incorporated in the lipid/PDA bilayer. This point is demonstrated when comparing the obtained detection limit using PCDA (10,12-pentacosadyinoic acid) functionalized with a LPS (lipopolysaccharide) aptamer (10^4 CFU/mL of *E. coli*) [30] and the obtained detection limit with SPH (sphingomyelin) incorporated in polyacetylene vesicle (10^0-10^1 CFU/mL of *S. choleraesuis*) [32]. The latter PDA vesicle-based assay was also tested for other types of bacteria. *P. aeruginosa* was also detected, as shown in Figure 5 [31], the selectivity of detection being closely dependent on the recognition molecule and also on the solution conditions (pH value) [31,32].

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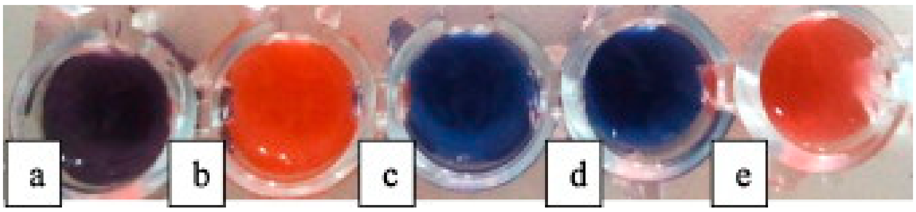

Figure 5: PCDA/SPH/Cholesterol/Lysine vesicles added to TSB (0.1%) and aqueous saline at pH 6.0 with (a) *E. coli*; (b) *P. aeruginosa*; (c) *S. aureus*; (d) *L. plantarum*; and (e) *S.Choleraesuis* (1×10^8CFU/mL). Reprinted with permission from [31]. Copyright 2017 Elsevier

Very rapid tests for the detection of bacteria in real samples were then designed using these mixed PDA vesicles for the detection of *Salmonella choleraesuis* in chicken meat [32].

Real time monitoring of the photocatalytic sterilization process in the presence of TiO_2 colloid was obtained by recording the colorimetric response (blue-red transition) of mixed polydiacetylene vesicles in the presence of *E. coli* (Fig. 6) [33].

Bacterial RNA was detected by fluorescence measurements through conjugation with on-chip immobilized PDA vesicles, previously grafted with complementary DNA probes. Different types of crude cell lysate (*E. coli*, *L. monocytogenes*, *S. enteritidis*) were incubated together with the specifically grafted vesicles. When target bacteria were matched with DNA probes, increased fluorescence intensities were observed. Although slight fluorescence corresponding to non-specific signal is detected with level of fluorescence much lower than that of matched probes. The detection limit was determined as 10^4-10^5 CFU/mL [16].

Table 1: PDA vesicles for detection of bacteria

Composition of the bilayer	Diameter of vesicles (µm)	Type of transduction	Type of bacteria	Bacteria LOD (CFU/mL)	Reference
TCDA/2% DGG		Colorimetry	*E. coli (ATCC25922)*	Not given	[25]
HCDA/DL3		Colorimetry	*E. coli*	10^8	[26]
PCDA-ABA/PCDA-biotin-streptavidin-anti *E. coli* antibody /(20-30%) DMPC		Fluorescence	*E. coli*	$1.2x10^7$	[27]
PCDA/glucose-tagged lipid or glucose-PCDA/rhodamine tagged DMPC	15-60 (GUVs)	Colorimetry Fluorescence (FRET)	*E. coli*	$3.3x10^5$	[28]
TRCDA/DMPC		Colorimetry	*E. coli*	10^8 (drinking water)	[29]
PCDA vesicles functionalized with LPS binding aptamer		Colorimetry	*E. coli (O157 :H7)*	10^4	[30]
PCDA/SPH/cholesterol/Lysine Lysine concentration 6.7 µg/mL, pH 6.5	0.2	Colorimetry	*S. choleraesuis*	10^8	[31]
PCDA/SPH/cholesterol/Lysine Lysine concentration 6.7 µg/mL, pH 6.0	0.2		*S. choleraesuis*	10^0-10^1 in chicken meat	[32]

DGG: dioctadecyl glycerylether-β-glucoside ; DL3: 3,6,9,12-tetraoxa-10-cholest-2-acetamido-2-desoxy-β-D-glucopyranoside ; DMPC: 1,2-dimysristoyl-*sn*-glycero-3-phosphatidylcholine ; GUVs: giant unilamellar vesicles ; HCDA: 2,4-heneicosadiynoic acid ; LOD: limit of detection ; LPS: lipopolysaccharide ; MLVs: multilamellar vesicles ; PCDA: 10,12-pentacosadyinoic acid ; PCDA-ABA: 10,12-pentacosadyinoic acid grafted with abscisic acid ; PCDA-biotin: 10,12-pentacosadyinoic acid grafted with biotin ; SPH: sphingomyelin ; TCDA: tricosa-2,4-diynoic acid ; TRCDA: 10,12-tricosadiynoic acid ;

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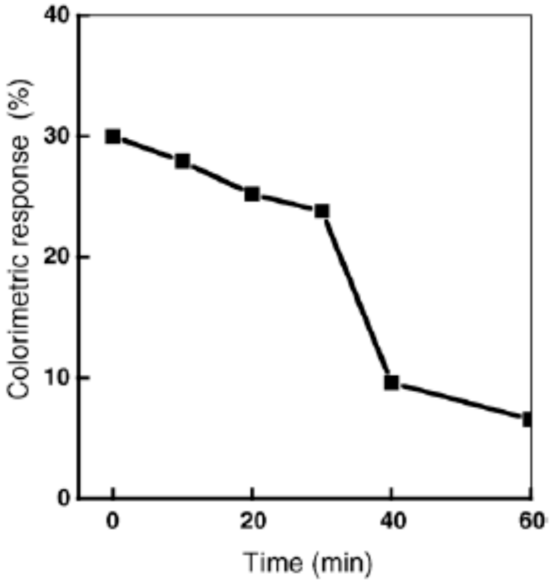

Figure 6: Colorimetric transition of mixed polyacetylene vesicles as a function of light irradiation time in the presence of TiO_2 in *E. coli* K12 suspension. Reprinted with permission from [33]. Copyright 2005 Elsevier

4.2. Indirect detection of hemolytic bacteria through toxin detection

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. PDA liposomes were synthesized in order to be able to detect this type of toxins through electrochemical or optical methods (colorimetry or fluorimetry) (Table 2). In order to mimic the cell membrane, the mixed bilayer is composed of a mixture of phosphocholine (DPPC, PC-DIYNE, DMPC) mixed with diacetylene monomer (GLY-PDA, TCDA, Gly-PCDA, PC-DIYNE) and cholesterol as a bait molecule since the first step for pore formation is believed to be toxin binding to cholesterol. For electrochemical detection, redox compounds such as ferrocene, hexacyanoferrate or 2,6-dichlorophenolindophenol, are entrapped in the vesicles [35-37] or inserted in bilayer membrane [34, 36]. Using ferrocene-PDA based vesicles, when the toxin is trapped by the receptor (ganglioside GM1), the toxin-receptor complex blocks the charge transfer route of the ferrocene probes to the electrode surface [34].

The optical techniques are based on the direct colour change of PDA through pore formation [38-40, 42, 45] or on the detection of released dye previously encapsulated in the vesicles [41, 43, 44] Different types of toxins were detected such as steptolysin O from *S. pyrogenes* and rhamsolipid from *P. aeruginosa*.

For streptolysin O, detected using amperometry via the redox probe (hexacyanoferrate) release, the obtained detection limit is 5 HU (hemolytic unit) [35] while detected by colorimetry

276 **Table 2:** PDA vesicles for detection of haemolytic bacteria and toxins

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Composition of the bilayer	Nature of the encapsulated probe	Type of transduction	Type of hemolytic bacteria	Type of toxin	Toxin LOD nM	Bacteria LOD (CFU/mL)	Reference
Electrochemical detection							
GLY-PDA/Fc-PDA/receptor ganglioside GM1	no	Amperometry	*E. coli*	*E. coli* Heat-labile enterotoxin	36		[34]
Phosphatidylcholine/cholesterol/ diacetyl phosphate/1-octadecanethiol	hexacyanoferrate	Amperometry	*S. pyrogenes A and C*	Streptolysin O	0.025 5 HU*		[35]
phosphatidylcholine	2,6-dichlorophenolindophenol	Amperometry	*L. monocytogenes* NCTC 7973	—	—	$5x10^6$	[36]
Phosphatidylcholine/2,6-dichlorophenolindophenol	no	Amperometry	*L. monocytogenes* NCTC 7973	—	—	$5x10^5$	[36]
DPPC/cholesterol/TCDA	hexacyanoferrate	Amperometry	*P. aeruginosa* PAO1 *S. aureus* USA300	Rhamnolipid Delta toxin	11000 29000		[37]
Optical detection							
Gly-PCDA/PC-DIYNE/cholesterol	no	Colorimetry	*S. pyrogenes A and C*	Streptolysin O	0.10 20 HU*		[38]
DMPC/10,12-tricosadiynoic acid	no	Colorimetric	*Salmonella enterica*	Bacterial supernatant		10^9 bacteria	[39]

Glycopolydiacetylene	no	Colorimetry	*E. coli* O157 :H7	Shiga toxin		1.2x10^{6}	[40]
DMPE /DMPE /TCDA/cholesterol	carboxyfluorescein	Fluorescence	*P. aeruginosa* PAO1 *S. aureus MSSA* 476			10^{4}	[41]
PCDA/TDER	no	Colorimetry	*S. aureus (ATCC 6538)* *E. coli (ATCC 11229)*	Bacterial supernatant		10^{8} (spiked apple juice)	[42]
DPPC /cholesterol/TCDA /DPPE	carboxyfluorescein	Fluorescence	*P. aeruginosa*	Rhamnolipid	40000	10^{6} CFU/mL	[43]
Hyaluronic acid/caprolactone	7-amino-4-methylcoumarin	Fluorescence	*S. aureus*	hyaluronidase	47 U/mL		[44]
Amine terminated PDA	no	Colorimetry Fluorescence	*B. subtilis, P. aeruginosa*	surfactin	16500	1.8x10^{3}	[45]

DMPC: 1,2-dimysristoyl-*sn*-glycero-3-phosphosphocholine ; DMPE: 1,2-dimysristoyl-*sn*-glycero-3-phosphoethanolamine ; DPPC: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine ; DPPE: 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine ; Fc-PDA: N-(10,12-pentacosadiunoyl)acetylferrocene ; Gly-PCDA: glycine-terminated diacetylene monomer ; GLY-PCDA: N-(10,12-pentacosadiunoyl)-glycine ; GM1: ganglioside ; LOD: limit of detection ; PCDA: 10,12-pentacosadyinoic acid ; PC-DIYNE: 1,2-bis(10,12-tricosadiynoyl)*sn*-glycero-3-phosphocholine ; TCDA: 10,12-tricosadiynoic acid ; TDER: N-[(2-tetradecanamide)-ethyl]-ribonamide

*HU (hemolytic unit) is defined as the amount of protein that causes 50% lysis of a 2% red blood cell suspension in PBS at pH 4.

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through blue-red transistion, the obtained detection limit is 20 HU [38], showing that the method of vesicle encapsulation leads to lower detection limits.

An intelligent hydrogel wound dressing based on fluorescent dye release from PDA vesicles was designed. The fluorescence allows early *in situ* detection of wound infection when haemolytic bacteria are produced in the wound (Figure 7) [46].

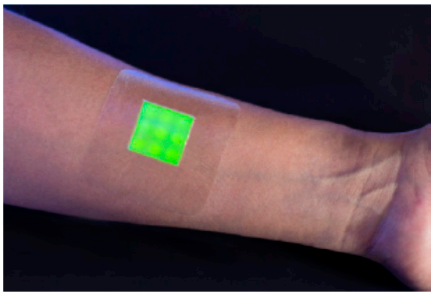

Figure 7: An intelligent hydrogel wound dressing based on fluorescent dye release from polydiacetylene vesicles. Reprinted with permission from [46]. Copyright 2016 American Chemical Society.

4.3. Screening of molecules with antibiotic properties

Assays providing rapid and easy evaluation of interactions between antimicrobial substances and PDA bilayer based vesicles as cell membrane model, could significantly improve screening of substances with effective microbial properties, as well as contribute to the elucidation of their structural and functional properties.

Due to their optical properties, polydiacetylene based vesicles were generally used for the design of these assays (Table 3). The first proof of concept was demonstrated for different antimicrobial peptides in Ref 47. The composition of the mixed bilayer was optimized in order to improve the blue-red transition in terms of intensity and response time. Lipopolysaccharide (LPS) was inserted to promote the interaction with antibacterial peptide [49, 50]. Lipid extracts from the red alga *Porphyridium cruentum* strain 1380.1/PDA vesicles were tested for the colorimetric detection of melittin and of polymixin B [51]. When these lipids present a lower total number of double bonds in the acyl residues, these antibacterial peptides induce higher colorimetric response, which might be due to higher fluidity within the lipid bilayer. Increased rigidity of the lipid moieties is expected to reduce penetration of the antibacterial peptide into the lipid bilayers, then resulting in peptide binding at the lipid headgroup region within the lipid/PDA vesicles. Such surface interaction is expected to induce greater perturbation of the pendant polymer side chains within the PDA matrix. Phospholipid vesicles inserting highly fluorescent dye allowed the very sensitive detection of alamethicin, an antibiotic peptide [48].

The antimicrobial properties of metabolites of soil microfungi were tested through a colorimetric assay using PDA-based vesicles [52]. This assay was also applied to the high throughput screening of peptides, bacteriocins, produced by lactic acid bacteria [12]. Figure 8 presents the percentage of colour change of DMPE/TRCDA vesicles treated with 50 µL cell-free supernatant of 54 lactic acid bacteria strains.

Table 3: PDA vesicles for detection of antibacterial peptides

Composition of the bilayer	Encapsulated probe	Type of transduction	Nature of antibiotic	Antibiotic LOD (μM)	Reference
DMPC/PCDA	No	Colorimetry	K7L-melittin Magainin II	100	[47]
POPC	Dipicolinic acid/Tb^{3+}	Fluorescence	alamethicin	0.25	[48]
LPS/DMPC/PCDA	No	Colorimetry	Indolicidin analog (prolines replaced by alanine)	30	[49]
LPS/DMPC/PDA	No	Colorimetry	Polymixin B derivatives	3	[50]
Lipid extracts from the red algae *Porphyridium cruentum* strain 1380.1/PCDA	No	Colorimetric	Melittin Polymixin B	1 1	[51]
DMPC/TCDA	No	Colorimetry	Antimicrobial membrane-active metabolites of soil fungi (strain 08-29-2)		[52]
DMPE/TRCDA	No	Colorimetry	Nisin Antibacterial peptides		[12]

DMPC: 1,2-dimyristoylphosphatidylcholine ; LOD: limit of detection ; LPS: lipopolysaccharides ; PCDA: PCDA: 10,12-pentacosadyinoic acid ; POPC: palmitoyl oleoyl phosphatidylcholine ; TCDA, TRCDA: 10,12-tricosadiynoic acid.

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It appears that for lactic acid bacteria strains LV39 (well 34), LB52 (well 40) and LB64 (well 47) the colour change is weak, compared to that of the other strains. These strains were considered to be bacteriocin non-producer and some of the other strains with colorimetric response higher than 50%, as bacteriocin producers.

PDA based liposome arrays for antibiotic detection, with PIP2 phospholipids as neomycin receptors, were schematized [53].

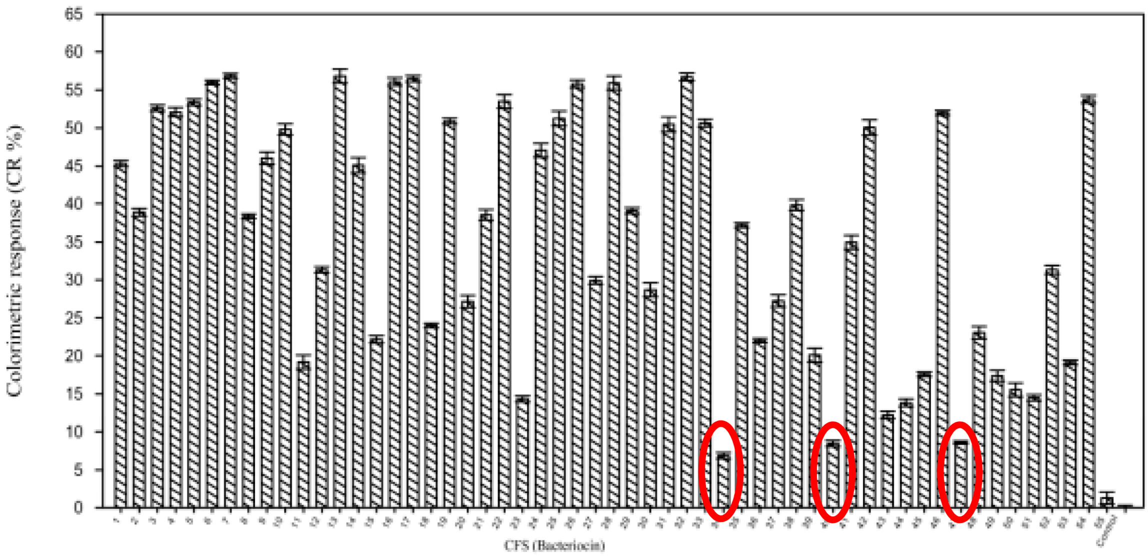

Figure 8: Colour change of DMPE/TRCDA vesicles treated with 50 μL cell-free supernatant of 54 lactic acid bacteria strains [12]

5. PDA vesicle based assays for detection of influenza viruses (Table 4)

Influenza virus particles are enveloped by a lipid bilayer to which the hemagglutinin (HA) lectin is anchored. HA binds to terminal a-glycosides of sialic acid on cell-surface glycoproteins and glycolipids, initiating cell infection by the virus. The same type of interaction was then biomimicked on the PDA vesicle surface. The first proof of concept of the direct detection of influenza viruses through a red-blue transition of sialic acid bound to functionalized PDA vesicles was described in 1995 [54], a detection limit of $11x10^7$ virus particles was obtained through colorimetric measurements. The same strategy was also proposed in Ref 55 and 56 and in Ref 57, sialic acid was grafted on a β-glucoside and sialic and lactose moieties were grafted on a glucoside chain for insertion in the PDA layer. Different other types of virus receptor were grafted onto PDA moieties: antibodies [58, 59] and peptides [60]. The analytical performance of the different approaches is very difficult to evaluate, because of the use of different units.

6. Conclusion and future directions

This review presents the state-of-the-art of mixed PDA based vesicles formulated in order to mimic cell membranes and how they constitute actual nanosensors for the direct detection of bacteria or

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viruses, of bacterial toxins (bacterial virulence) and of antibacterial and antiviral peptides, through direct blue-red transition or through the passive release of encapsulated probes.

The colorimetric assays based on these PDA based vesicles are very cheep and easy to handle. They were applied to the high throughput screening of toxins of natural origin (fungi [52], bacteria [12]) through the design of a biosensing platform [12]. They were also applied to rapid bacterial detection in food [32, 39].

It has been observed that the vesicle encapsulation method, leading to an amplification effect, provides lower detection limit, through the electrochemical detection of redox probes or the optical detection of fluorescent probes. Improving vesicle stability, minimizing passive leakage of encapsulated probes, developping functionalized vesicles for the specific detection of pathogens are the main challenges for improving the vesicle encapsulation method for biosensing.

However, there are still important bottlenecks that limit the development of PDA vesicle-based bioassays. In aqueous solution, the sensitivity of PDA vesicles is usually unsatisfactory for applications in medical diagnostic, food safety…. For increasing the color change, a high concentration of analyte is required. The reversibility of PDA vesicle-based bioassays is also attractive for obtaining a reusable sensor. Reversible responses can only be achieved by heating, pH change, UV light… and no PDA sensors for biochemical analytes are reversible. Rigorous theoretical studies and simulations of the important transitions, leading the colour change are required in order to develop a clearer understanding of their origin. Improvement of stability under various conditions is a major concern, particularly rehydratation efficiency to enable dry sensor forms to be developed that would be a necessary step towards the potential commercialization of point of care systems based on microarrays of PDA vesicles. Some cryoprotectant could be used for this purpose [61].

The ultimate solution to all these issues will enable development of highly sensitive PDA vesicle-based biochips for convenient rapid tests for medical diagnostic, food safety…. that could become IoT systems.

Table 4: PDA vesicles for detection of viruses

Composition of the bilayer	Diameter of vesicles	Type of transduction	Type of virus	Virus LOD	Reference
95% PCDA/5% sialic acid derivatized PCDA		colorimetry	Influenza	11×10^7 virus particles	[54]
95% PCDA/5% sialic acid derivatized PCDA		colorimetry	Influenza	8×10^7 virus particles	[55]
PCDA/5% S-sialo PCDA		colorimetry	Influenza X-31	0.78 HAU	[56]
PDMA/DMPC/G1/G2 G1 : sialic acid-β-glucoside G2 : lactose-β-glucoside	10-20 nm	colorimetry	H5N1 Avian influenza	10 ng/mL hemagglutinin	[57]
PCDA Anti-H5N1 monoclonal antibody grafted on vesicle surface	117 nm	colorimetry fluorescence	H5N1 Avian influenza	30 ng/mL HAQ 1 ng/mL HAQ	[58]
PCDA/DMPC Anti-HA monoclonal antibody grafted on vesicle surface		colorimetry	H5 influenza	0.53 copies/µL	[59]
PEP-PCDA	~ 50 nm	colorimetry	H1N1 influenza	10^5 PFU	[60]

DMPC: dimyristoylphosphatidylcholine ; HAQ: target antigen of H5N1 Avian influenza virus strain ; LOD: limit of detection ; PCDA: 10,12-pentacosadiynoic acid ; PEP-PCDA: peptide-functionalized 10,12-pentacosadiynoic acid

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