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Liposome-based nanosensors for biological detection

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Abstract: Liposomes are self-assembled structures that contain an inner aqueous compartment surrounded by a lipid bilayer. This unique structure inherently provides liposomes with a powerful capability for encapsulating hydrophilic, hydrophobic or amphiphilic molecules or nanoparticles. Combining this property with appropriate signal amplification strategies and transduction techniques results in a variety of in vitro or in vivo biological sensors. In this review article, we discuss the latest trends in engineering and applications of liposome based nanosensors for biological sensing. Particular focus was made on the coupling of liposomes with popular sensor materials (enzymes, quantum dots, metal nanoparticles and other sensor enhancement elements) for highly sensitive and selective detection of chemical and biological species. Such information will be viable in terms of providing a useful platform for designing future ultrasensitive liposome nanosensors.

Keywords: Liposome, Sensor, Nanotechnology, Lipid Bilayer, Ultrasensitive, Biological, Encapsulation

1. Introduction and Background

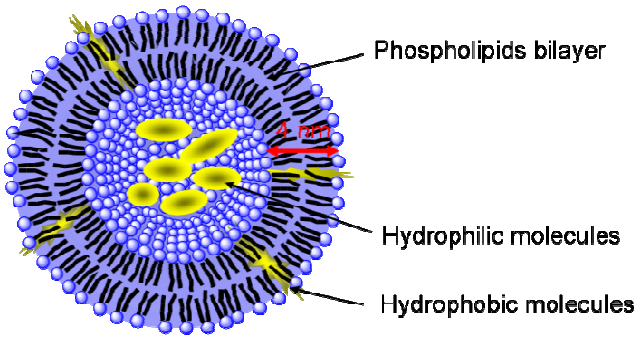

Fig. 1. *A schematic drawing of a unilamellar liposome. Hydrophilic molecules can be entrapped inside the liposome, while molecules with hydrophobic portions are oriented within the membrane.*

Liposomes are self-assembled structures that contain an inner aqueous compartment surrounded by a lipid bilayer typically composed of phospholipids and sterols. Since it was first discovered by Bangham[1] in 1965, Liposomes are widely used as model systems for cell membranes and as drug carriers in drug delivery systems[2]. Liposomes can be easily prepared by the hydration of lipid thin film followed by downsizing using extrusion or sonication techniques. As described in figure 1, liposomes can simply be modified in a desired manner through the choice of membrane components and it is this property that has made them attractive as model systems for cell membrane. For example, using newly developed IR-based spectroscopy methods in combination with spherical liposomes formulated with different lipids and sterols, Chen et.al. studied the structure-property relationships of liposomes such as membrane fluidity[3], transport [2] and lysis [4]. Furthermore, liposomes possess a unique structure, possessing both a hydrophilic interior and exterior, and a hydrophobic region within the lipid membrane. Such a structure makes it possible to either encapsulate water-soluble molecules in the hydrophilic interior of the liposome or immobilize molecules within the lipid membrane. As a result, liposomes are frequently used as carriers of chemicals, biomolecules and nanoparticles, finding applications in drug delivery, chemical and biological sensors. It is also possible to target specific cells by attaching an appropriate molecule to the liposome surface that binds specifically to a receptor site[5, 6].

Because of this unique structural advance, liposomes also received considerable attention for use as a substrate in sensors for chemical and biological detection. The liposome, combined with effective transduction technology including fluorescence, calorimetric, and optical spectroscopy, could realize enormous signal amplification and achieve ultrasensitive assays. The major functions of the liposomes in

these sensors include the following:

1. Working as carrier for active sensor materials or sensor enhancing elements for signal amplification or multi-target detection when carrying multiple sensor materials.
2. Stabilizing the activity of enzymes or proteins for selective detection.
3. Improving the biocompatibility and accessibility of the sensors in biological medium.

This review article is intended to promote the awareness of the function of liposomes in selective and sensitive nanosensor development, and outline the current status and potential applications of liposome-based sensors in environmental sensing and biological screening. It discusses key functions of liposomes when coupling with other sensor materials (enzymes, quantum dots, metal nanoparticles, etc) on current sensor systems for biological sensing. This discussion will encourage the biological sensor community to think about possible applications of liposome-based sensors such as chemical/biological agent detection, early disease diagnosis, and biological species screening.

2. Liposome-Enzyme Based Biosensor

In recent years, because of their green nature, high selectivity, and sensitivity, the use of enzymes in biological sensors received increasing scientific attention. However, current continuous enzyme-based sensing is limited by the instability of most enzymes and their sensitivity to changes in the environment. Enzymes are inherently unstable and tend to lose activity due to folding in free solution. One major challenge in developing enzyme-based biosensors is the stabilization of the enzyme. Many approaches have been developed including the immobilization of enzyme on substrates[7-9] and polymer materials[10]. One promising approach is to protect enzyme from deactivation by encapsulating the enzyme within a liposome membrane or inner aqueous cavity. It has been proven that liposomes can stabilize enzymes in their nano-cavities against unfolding, denaturation and dilution effects due to the hydrophobic force between the enzyme and lipid bilayers[11-13]. This improves the long term stability of enzyme which is critical in biosensor applications.

Vamvakaki and Chaniotakis [14] have developed a liposome-enzyme based nanobiosensor for organophosphorous pesticide detection in drinking water systems. This sensor utilized liposome stabilized enzyme acetylcholinesterase in combination with fluorescence techniques as the optical transduction scheme for trace level detection of dichlorvos and paraoxon. As shown in Figure 2, the inherently unstable enzyme acetylcholinesterase and pH sensitive fluorescence indicator pyranine were encapsulated in eggPC liposomes with an average diameter of 300 nm. Also, Porin was embedded in the liposome membrane to control the selective transport of acetylcholine substrate into the liposomes. The controlled diffusion of the substrate inside the liposome initiates the enzymatic hydrolysis reaction which leads to the production of acetic acid and thus to pH decrease at the local nano-environment of the enzyme. This pH decrease is monitored by a decrease of pyranine fluorescence signal, which can be subsequently correlated to the substrate concentration. Exposing the nano-biosensor to organophosphorous pesticides would block the enzyme activity and decrease the production rate of acetic acid and thus the pH change. As a result, by monitoring the response of the pH sensitive fluorescence indicator signal in the presence of pesticides, the concentration of organophosphorous pesticides can be determined.

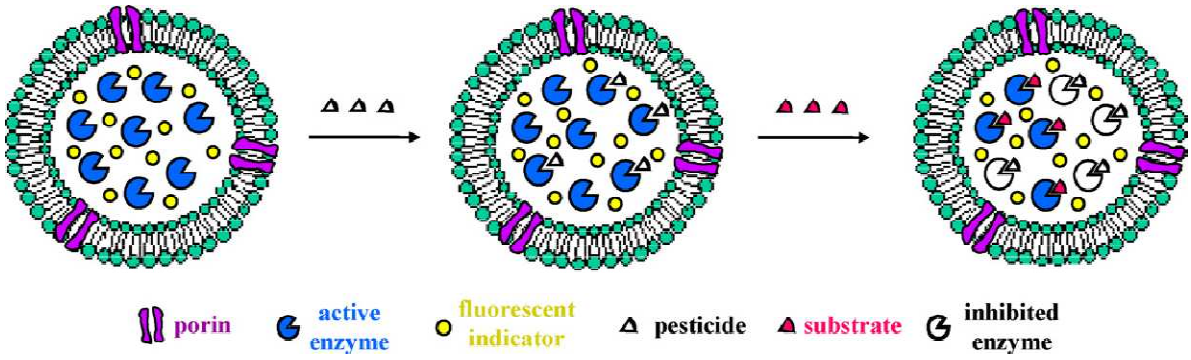

Fig. 2. *Schematic diagram of the AChE-based inhibitor liposome biosensor. The scheme was reproduced with the permission of Elsevier.*

In this work, the stabilization effect of the liposome on the nano-biosensor was proven by comparing the storage stability of the AChE/liposome nanobiosensor and enzyme in free solution. The liposome encapsulated enzyme retained full activity even after 50 days storage in ambient conditions while the free enzyme lost 66% of its original activity towards acetylcholine substrate. In addition, a detection limit of 10^{-10} M of dichlorvos and paraoxon was achieved by the AchE/Liposome nanobiosensor.

3. Liposome-Quantum Dot Complexes for Single Particle Detection

Semiconductor quantum dots (QDs) since its discovery in 90'[15] immediately draw the attention of researchers in sensor field. Because of their superior optical properties such as broad excitation, narrow emission, high quantum yield, and photochemical stability[16, 17], they become excellent

alternatives for organic fluorescent dyes in biomedical research, biological labeling[18, 19], and in vitro/in vivo imaging[20]. A combination of quantum dots with single particle detection techniques has been widely used in DNA sensing[21] and has shown an advantage of a high signal-to-noise ratio, improved sensitivity and low sample consumption. However one major limitation for conventional QD-based nanosensors is that signal enhancement relies on the assembly of multiple target molecules on the surface of a single QD and thus the sensitivity is limited by the availability of both the target molecules and the QDs[22-24].

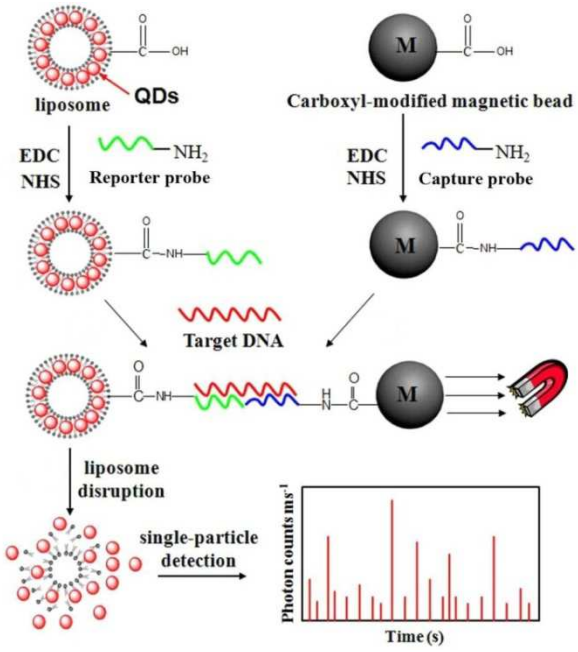

Fig. 3. *Schematic illustration of DNA sensing by using liposome-QDs complexes in combination with a single molecular detection technique. This scheme was reproduced with the permission of ACS publications.*

To overcome this limitation, Zhou et.al[17] reported a new approach using liposome-quantum dots complexes in combination with single particle detection techniques for sensitive detection of attomolar DNA. As shown in figure 3, liposomes were used to encapsulate QDs to form liposome/QDs complexes. The carboxyl-functionalized L/QD complexes and carboxyl-modified magnetic beads were covalently conjugated with the amino-terminated oligonucleotides producing the reporter probes and the capture probes, respectively. In the presence of target DNA, a sandwich hybrid structure consisting of a reporter probe, a capture probe and the target DNA was formed. After separating them from the free report probe using magnetic beads, the liposome/QDs were disrupted and the release of QDs was subsequently measured by single particle detection. The advantage of using the lipsome-QDs complex can be summarized as the following: first of all the use of liposomes greatly improves the detection sensitivity by encapsulating hundreds of QDs. Second, the unique nature of liposomes provides accessible functionality and desirable biocompatibility for biomedical applications. In addition, simultaneous detection of multiple DNA targets can be easily realized by using L/QD complexes with different colors.

4. Liposome-Gold Nanoparticle Nanocomposite

Gold nanoparticles, because of their efficient optical properties and ease of surface functionalization[25], have been widely used in biological detection[26, 27] including DNA hydridization, DNA-ligands, DNA-protein interactions and cell transfection, etc.. Because of the broad application of liposomes in drug encapsulation as well as DNA transfection, a variety of liposome-metal nanoparticle composites were produced and widely used as biological sensing substrates.

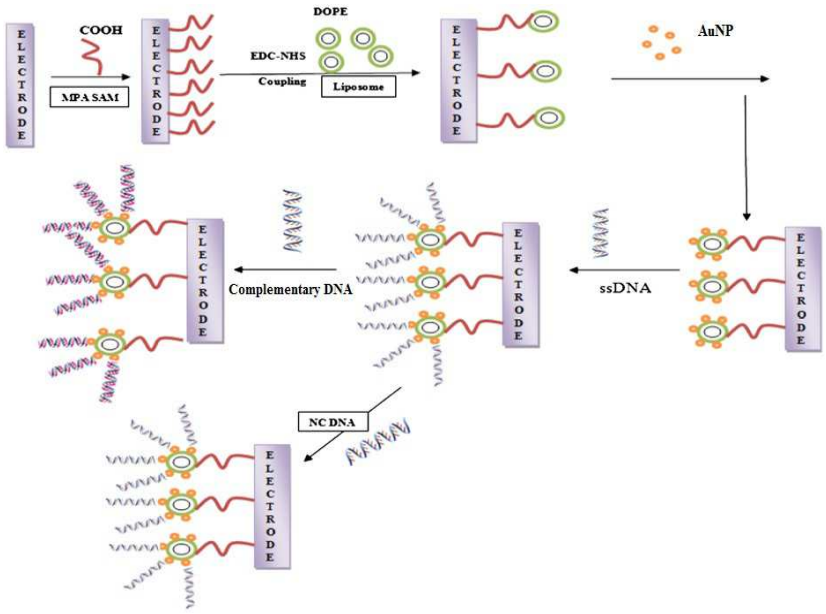

Fig. 4. *Schematic illustration of the fabrication of DOPE-AuNPs nanocomposite and DNA detection. This scheme was reproduced with the permission of Elsevier.*

Bhuvana et. al.[28] reported liposome-gold NPs nanocomposites that were immobilized on a solid electrode for in situ electrochemical DNA sensing. As described in figure 4. The DOPE liposomes were first immobilized on the gold electrode through covalent bonding with 3-mercaptopropionic acid (MPA) on gold surface. Next the liposome-AuNPs nanocomposites were formed by electroless deposition of AuNPs (4.58 nm) on the amine headgroup of DOPE liposomes. Single stranded probe DNA, were then immobilized on the AuNPs surface through the well-known gold-thiol bond. The resulting sensing substrate in combination with Cyclic voltammetry (CV), electrochemical impedance (EIS), differential pulse voltammetry (DPV) and quartz crystal microbalance (QCM) techniques showed high selectivity and sensitivity for DNA. The limit of detection was determined to be 0.1 femtomolar. The use of liposomes increases the bio-relevance as the liposome-AuNPs nanocomposites are fully exposed to the solution, unlike traditional AuNPs immobilized electrode in which the substrate has limited access to the solution. On the other hand, the abundance of AuNPs on the liposome surface improved the signal-to-noise ratio for DNA sensing.

5. Other Immunoassays Using Signal Enhancer Molecular Encapsulated Liposome

Because of its unique spherical bilayer structure in aqueous systems, liposomes are able to carry hydrophilic, hydrophobic and amphiliphic molecules and release the content upon exposure to surrounding stimulation. Based on this, a variety of biosensors were developed using encapsulation and release of signal enhancer molecules in liposomes. Damhorst, et. al.[29] reported a liposome based ion release impedance sensor for biological detection at the point of care. The core component of the sensor is a micron-sized antibody surface functionalized liposome encapsulating concentrated phosphate buffer saline (PBS). PBS buffer ions were used as the sensor component as they are of significantly low permeability in liposomes in ambient conditions. Chen and Tripp[2] have studied the permeability of different sizes and types of molecules in liposomes and showed that ionic molecules have the lowest release rate. The proof-of-concept experiments were carried out in a microfluidic device for HIV detection. The microfluidic device was pre-functionalized with anti-gp120 antibody and then exposed to HIV virus solution. After sufficient incubation, the unbound HIV virus was removed and IgG-functionalized liposomes in PBS were injected through the device. The unbound liposomes and free PBS ions in the solution were immediately removed by rinsing with DI water. The device was then heated for promoting the release of PBS ions from the liposome, and the impedance was monitored and compared to the control experiment with a virus-free environment. Significantly larger changes in the impedance after liposome injection in the virus-containing device suggested the capture of liposomes on immobilized HIV virus. The number of HIV viruses can be calculated by the number of bound liposomes which are subsequently calculated using normalized impedance change. Similar detection strategies provide a simple and low cost solution for biological sensing at the point of care.

Using a similar strategy, Mao et.al[30] developed a liposome-based electrogenerated chemiluminescence (ECL) immunoassay for the detection of heart failure biomarker N-terminal pro-brain natriuretic peptide (NT-proBNP). In this detection strategy, cocaine was used as the signal enhancer molecule to enhance the ECL of $Ru(bpy)_3^{2+}$. $Ru(bpy)_3^{2+}$ was encapsulated in the liposome and released for ECL measurement. They specifically designed a sandwich immune sensing platform for the experiments. At the beginning, the glassy carbon electrode (GCE) was surface-modified by the electrodeposition of gold-platinum nanoparticles and then post-modified with capture antibodies (mAb1). The modified electrode was then incubated in the solution of antigen of the NT-proBNP biomarker to load the biomarker. Next the electrode was incubated with a secondary capture antibody (mAb2) functionalized with cocaine encapsulated liposome to allow the liposome to bond to the GCE-mAb1-NT-proBNT hybrid. In the detection procedure, the liposome based sensing sandwich was treated with triton X-100 to release the cocaine which was subsequently detected by the Ru-based ECL aptasensor. With increasing NT-proBNP concentration, the conjugated liposomes increased on the object-electrode, and thus the released cocaine increased as well. This led to the enhancement of the Ru-based ECL intensity and the changes of ECL intensities can be correlated with the concentration changes of NT-proBNP. The core detection strategy in this sensor is that encapsulation in liposomes can increase the amount of the enhancer molecule cocaine, which can multiply the ECL signal response and achieve an ultrasentive assay. The reported NT-proBNP assay exhibited high sensitivity with a linear relationship over 0.01–500 ng/mL range, and a detection limit of 0.77 pg/mL.

Summary

Because of the unique lipid bilayer structure, liposomes showed superior advantages over other biostructures in biosensor fabrication such as excellent biocompatibility, easy preparation and modification. The encapsulation or surface attachment of sensor materials and subsequent release of liposome contents provide a simple and effective approach for signal amplification and transduction. It is ready to be compatible with current sensor technology including semiconductor quantum dots, nanoparticle, immunoassay, electrochemical, fluorescence and optical spectroscopy, etc.. In addition, by incorporating different sensor materials and target molecules, liposome based sensors can easily be modified for multitarget detection and sensing.

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