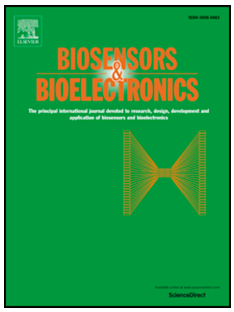

A soft metal-polyphenol capsule-based ultrasensitive immunoassay for electrochemical detection of Epstein-Barr (EB) virus infection

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A soft metal-polyphenol capsule-based ultrasensitive immunoassay for electrochemical detection of Epstein-Barr (EB) virus infection

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Abstract

Herein, we have proposed a soft metal-phenolic capsule (sMPC)-based electrochemical immunoassay for ultrasensitive detection of Epstein-Barr virus capsid antigen IgA (EBVCA-IgA), a biomarker of nasopharyngeal carcinoma. Metal probes with large size contain a number of metal ions, which are very beneficial to signal amplification for anodic stripping voltammetry; however, these probes easily precipitate due to their heavy weight, leading to low recognition efficiency and compromised performance. In this study, we demonstrate sMPCs fabricated by metal-coordination interactions exhibit unique surface behavior compared with their solid counterparts, which significantly enhance recognition efficiency and thus improve sensitivity despite of their micrometer size. Taking advantage of the sMPCs, the involved electrochemical immunoassay shows a much-improved sensitivity with an ultralow detection limit of 0.46 fM for EBVCA-IgA and can also be used in real sample analysis. So far as we know, this is the first report on a sMPC-based electrochemical strategy. Furthermore, it clarifies the potential effect of the rigidness of probes on the performance of an involved biosensor, which is meaningful to guide the design of other functional probes. The advantages of this method, including easy to fabrication, ultrasensitivity and good selectivity, ensure a promising potential in the point-of-care diagnostics of critical diseases.

Key words: anodic stripping voltammetry; electrochemical sensor; metal-phenolic capsule; probe rigidness

1. Introduction

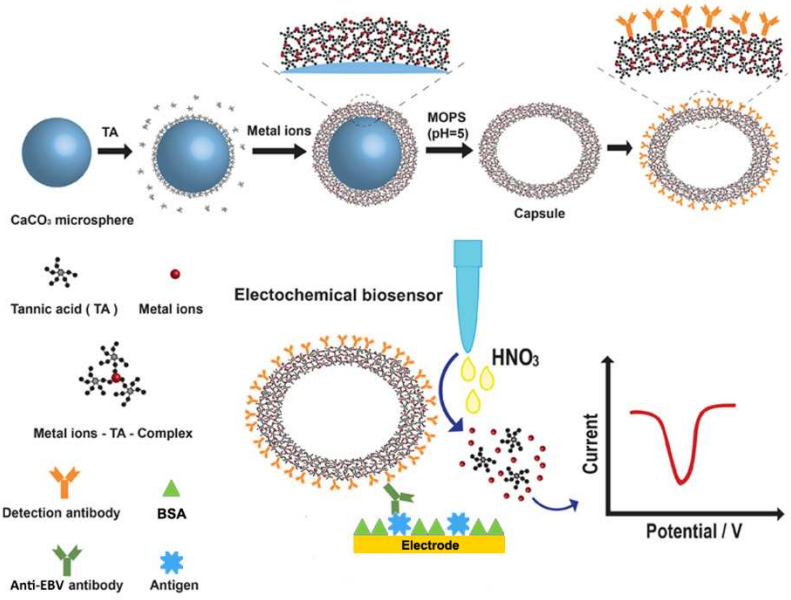
Nasopharyngeal carcinoma (NPC) is an epithelial cancer originating from the mucosal lining of nasopharynx, and is especially endemic in east and Southeast Asia. It is reported that the age-standardized incidence of NPC is 3.0 per 100000 in China and the 5-year overall survival rate is 41%.(Chen et al. 2019) Epstein-Barr virus (EBV) has been identified as a prominent pathogenic factor NPC,(Dai et al. 2016; Li et al. 2017; Zheng et al. 2016) so effective population screening of EBV plays a significant role in early-stage NPC diagnosis. Analysis of plasma EBV DNA using PCR has been regarded as an effective method for EBV detection, but the results vary distinguishingly between laboratories due to lack of a standardized assay. Recently, anti-EBV antibodies serological testing has been developed developed to be one of the routine screening of NPC. However, conventional antibody detection based on enzyme-linked immunoassay (ELISA) suffers from low sensitivity and specificity. Therefore, there is an urgent demand for highly sensitive, specific and stable methods to detect anti-EBV antibody.

Electrochemical biosensors convert biochemical information, such as analyte concentration into an electronic readout (e.g. current or voltage). So far, multiple electrochemical techniques are applied in biomedical fields for disease biomarkers detection because of their attractive merits such as high sensitivity, inherent simplicity, fast response, and low cost.(Amani et al. 2018; Ghanbari et al. 2019a; Rahimi-Nasrabadi et al. 2017a; Rahimi-Nasrabadi et al. 2017b; Wang et al. 2019; Yang et al. 2019) Among them, anodic stripping voltammetry (ASV) is one of the most extensively used electrochemical techniques with impressive sensitivity (typically 10^{-9} - 10^{-10} M) for detection of metal ions. Hence, preparation of proper metal probes to produce amplified stripping current has been a popular method for construction of ultrasensitive electrochemical sensors. For example, Wan and co-workers developed an ASV biosensor based on a family of antibody/DNA-labeled metal nanoparticle (MNP) labels for multiplexed identification of cancer cells.(Wan et al. 2014) Chen et al. constructed nanoscale aptamer/metal organic framework (NMOF)-based probes for electrochemical detection of antibiotic based on the high metal loading capacity of NMOF.(Chen et al. 2017) Zhang and colleagues used quantum dots-based method to detect telomerase at the single-cell level, in which the released Cd (II) ions from QDs were quantified by ASV.(Li et al. 2018) However, design of a metal probe leveraging the effectiveness of both molecular recognition and signal amplification remains a great challenge, since most of the used metal probes have a small size range (from 10 nm to 100 nm) that contains limited number of metal ions, which is not suitableto signal amplification.

Generally, the amount of metal ion confined in one metal probe is directly associated to its size, and therefore, metal probes with large diameter should be particularly suitable for ASV analysis.(Shang et al. 2019; Zhang et al. 2019) Nevertheless, inevitably, large and solid metal probes rapidly deposit on the sensor surface because of their heavy weight, which reduces the recognition efficiency and leads to false-positive signal.(Ghanbari et al. 2019b; Khoshroo et al. 2019) Naturally,

leukocytes with the size of serval micrometer can freely roll along the blood vessel without any adhesion, because they are soft, capsule-like structures. During their movement, leukocytes may contact with inflamed epithelial cells on the blood cells. Then, the specific interaction between adhesive molecules on epithelial cells and transmembrane receptors on the leukocytes can recruit more white blood cells to injured or pathologic tissues.(Jeong et al. 2013) Inspired by the recognition manner of leukocytes,(Moshaei et al. 2019) we hypothesized that a soft metal particle may be an ideal candidate for ASV-based analysis despite of its large size, because it may slowly roll rather than rapidly precipitate on the solid surface, which provides more useful contact area and improved orientation between antigen and antibody, thus enhancing adhesive chance toward its analytes on surface.

Recently, metal-phenolic complexes have aroused much attention due to their outstanding features of simple synthesis, high mechanical and thermal stability, controllable surface chemistry, and pH-responsive assembly/disassembly.(Ejima et al. 2017; Ejima et al. 2013) To do so, tannic acid (TA), a natural polyphenol, is selected as the organic ligand because its galloyl groups can form a cross-linked film with different metal ions. Caruso and other groups developed many types of MPCs and made use of them to perform different tasks such as catalysis,(Song et al. 2015; Zeng et al. 2014) cell imaging,(Liang et al. 2016) drug delivery,(Guo et al. 2014; Ping et al. 2015) and antifouling.(Ju et al. 2015; Kim et al. 2015) However, to the best of our knowledge, taking advantage of MPCs as an effective metal probe for electrochemical biosensor has never been explored. In addition, the behavior of MPCs on a solid surface is also seldom studied.



Scheme 1. The schematic illustration of the principle of the proposed metal-phenolic capsules-based electrochemical immunoassay. Note that the figure is not drawn to scale.

Herein, we describe a novel electrochemical strategy for anti-EBV capsid antigen antibody (EBVCA-IgA) detection based on a soft metal-phenolic capsules (sMPC). The

main principle of the electrochemical immunoassay is shown in Scheme 1. The preparation of metal-phenolic (MP) capsules is direct and simple and the whole process can be completed within 30 min. Briefly, metal ion and TA was mixed and immediately incubated with micro-sized calcium carbonate ($CaCO_3$) microspheres. Because of the high affinity between polyphenol and metal ions, metal-phenolic network formed and deposited on the $CaCO_3$ microspheres. Then, the pH value of the solution was adjusted to 5.0 to dissolve the template. The soft and hollow capsules were collected by centrifugation and incubated with anti-human IgA to form the detection metal probe.

Our results revealed that sMPC exhibited leukocyte-like movement behavior on the electrode surface. Therefore, it had better analyte-recognition efficiency compared with its solid counterpart. Once formation of a classic sandwich structure on the electrode surface, the sMPCs were dissolved by adding HNO_3 and the released metal ions could be used as signal molecules. Each sMPC contains a tremendous number of metal ions, so amplified current signal can be detected by ASV, which allows the ultrasensitive detection of tumor biomarker.

2. Experimental section

2.1 Chemicals and instruments

Calcium chloride anhydrous ($CaCl_2$), copper (II) chloride dihydrate ($CuCl_2$), iron trichloride ($FeCl_3$), potassium chloride (KCl), nitric acid (HNO_3) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Sodium carbonate anhydrous (Na_2CO_3) and poly(sodium-*p*-styrenesulfonate) (PSS) were obtained from Macklin (Shanghai, China). Tannic acid (TA) and 3-Morpholinopropanesulfonic acid (MOPS) were purchased from Aladdin (Shanghai, China). Lead (II) acetate ($PbAc_2$) and zinc chloride ($ZnCl_2$) were purchased from Sangon Biotech (Shanghai, China). Antibodies were ordered Co., Ltd (Shanghai, China). Phosphate buffered saline (PBS, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) was purchased from Sangon Co., Ltd (Shanghai, China). All the chemicals and reagents were of analytical grade and used without further purification, unless otherwise noted. All the solutions and subsequent dilutions are made using Milli-Q water.

All electrochemical immunoassay measurements were performed on a CHI 760E Electrochemical Workstation (Shanghai, China). A gold electrode (3 mm diameter) was used as working electrode, with Ag/AgCl and platinum wire acted as the reference electrode and auxiliary electrode, respectively. The images were captured by an optical microscopy (Olympus IX71, Japan). The transmission electron microscopic (TEM) image was obtained with a JEM-1400 flash transmission electron microscope (JEOL, Japan). Scanning electron microscope (SEM) image was obtained with a S3400N scanning electron microscope (Hitachi, Japan). Atomic Force Microscope (AFM) image was obtained with a Dimension Icon atomic force microscope (Bruker, Germany). The size distribution of nanoparticles was measured by a dynamic light-scattering (DLS) method using a Zeta-sizer Nano ZS (Malvern Instruments, United Kingdom). The particle count was determined by an Accuri C6 flow cytometer (BD Biosciences, USA).

2.2 Synthesis of antibody-modified metal-phenolic capsules (Ab-MPC)

The synthesis of metal probe was performed according to a previously reported method with some alterations by using $CaCO_3$ microparticles as the sacrificial template and Pb (II) and TA as the network substrate.(Ejima et al. 2013) For the fabrication of the sacrificial template, 5 mL of a 0.2 M $CaCl_2$ solution with 4 mg/mL PSS and were slowly dropped into an equal volume of 0.2 M Na_2CO_3 solution with 4 mg/mL PSS. The reaction mixture was lasted for 30 min by vigorous stirring. The mixture was centrifuged and the sediment was washed 3 times with Milli-Q water. Then, $CaCO_3$ microparticles were suspended in Milli-Q water.

Aliquots (10 μL) of TA (10 mM) and $PbAc_2$ (10 mM) solutions were simultaneously added to the 80 μL aqueous $CaCO_3$ microparticle suspension to yield the following final concentrations ($CaCO_3$: 1 mg/mL, $PbAc_2$: 1 mM, and TA: 1 mM). Afterwards, the suspension vortex vigorously for 10 s. After 5 min of incubation, $CaCO_3$ microparticles were centrifuged (500 rpm, 10 s) and washed thrice with Milli-Q water to remove excess TA and $PbAc_2$ in the supernatant. Afterwards, the TA/Pb-coated microparticle templates were resuspended in MOPS (pH = 5.0) for 15 minutes with gentle pipetting to dissolve the $CaCO_3$ core. And then, the suspension was centrifuged again (3000 rpm) and the resulting capsules were resuspended in HEPES buffer (pH 7.4). 1 μL of antibody solution (2 mg/mL) was incubated with 99 μL of the as-synthesized capsules for 1 h with slightly shaking and the unbound sites were blocked by BSA (5 mg/mL), and the redundant proteins were removed by centrifugation. The final antibody-modified capsules were stored at 4 °C for further use.

The number of metal ions in one particle was estimated by following steps. 100 μL of the as-synthesized FITC-labeled antibody (FITC-Ab)-modified capsules were counted by flow cytometer. Then, the same volume of capsules was totally dissolved by nitric acid and the concentration of metal ion was measured by inductively coupled plasma mass spectrometry (ICP-MS). Finally, the number of metal ion in one particle can be estimated.**2.3 Electrochemical detection of EBVCA-IgA**

The gold electrodes were cleaned according to our previously reported methods.(Ma et al. 2019) Then, the resulting electrodes were directly incubated with EBVCA (100 μg/mL) for 2 h at room temperature. After washing, BSA (10 mg/mL) was used as blocking agent to passivate the blank sites of the electrode surface. After washing with PBS containing 0.05% Tween-20 (PBST) buffer, the electrodes could be stored at 4 °C for further use.

The analyte solution (10 μL) was incubated with the antigen-modified electrode for 1 h at 37 °C. After thoroughly washing, the electrodes were incubated with as-prepared sMPC solution with gentle shaking at 150 rpm for 0.5 h at 37 °C. After washing, 5 μL of HNO_3 (0.01 M) was coated on the electrode to destroy the adsorbed metal probes and 95 μL HAc-NaAc (10 mM, pH 4.5) buffer solutions containing KCl (100 mM) was introduced as the electrolyte. Deposition of the metal ions was performed at a constant potential of -1.0 V for 90 s. Then, a square wave voltammetry (SWV) measurement was carried out from −1.0 V to 0 V, 25 Hz frequency, and a potential step of 4 mV with pulse amplitude of 25 mV. The current responses were associated with the analyte concentrations.

Serum samples from healthy individuals and patients were obtained from local hospital. The sera were tested for presence of anti-EBV antibodies with commercially available ELISA kits.

3. Results and Discussion

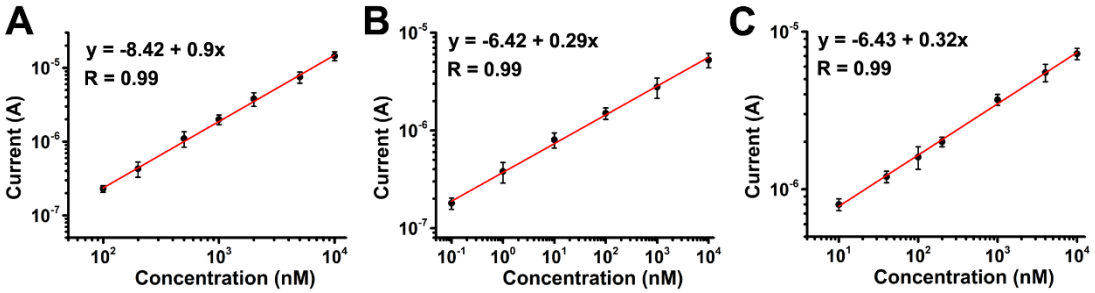


Figure 1. ASV response of (A) Cu^{2+}, (B) Pb^{2+} and (C) Zn^{2+} with different concentrations using gold electrode. Electrolyte is 10 mM NaAc-HAc buffer (pH 4.5) containing 100 mM KCl.

3.1 Electrochemical detection of metal ions using gold electrode

Previously, mercury film electrode was extensively used in ASV analysis; however, the coating of highly toxic mercury film on electrode needs careful operation and may be harmful to environment. In this study, we tried to use the commonly used gold electrode for ASV analysis through systematically investigating the electrochemical response of different metal ions such as Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, Cr^{2+}, Fe^{3+}, Y^{3+}, Al^{3+}, and Zr^{4+} in 10 mM HAc-NaAc buffer (pH 4.5). As shown in Figure S1, only Cu^{2+}, Pb^{2+} and Zn^{2+} produced large and well-defined anodic peak at 0.23 V, -0.16 V and -0.52 V on gold electrode, respectively, so they were further used for electrochemical determination. Figure 1 shows the titration curves of Cu^{2+}, Pb^{2+} and Zn^{2+} at different concentrations. It is clearly seen that Pb^{2+} produces the most sensitive electrochemical signals, which the detection limit for a 90 s electrodeposition was 0.1 nM without removal of oxygen (Figure S2). Therefore, Pb^{2+} has been used for further experiment.

3.2 Characterization of the prepared sMPCs

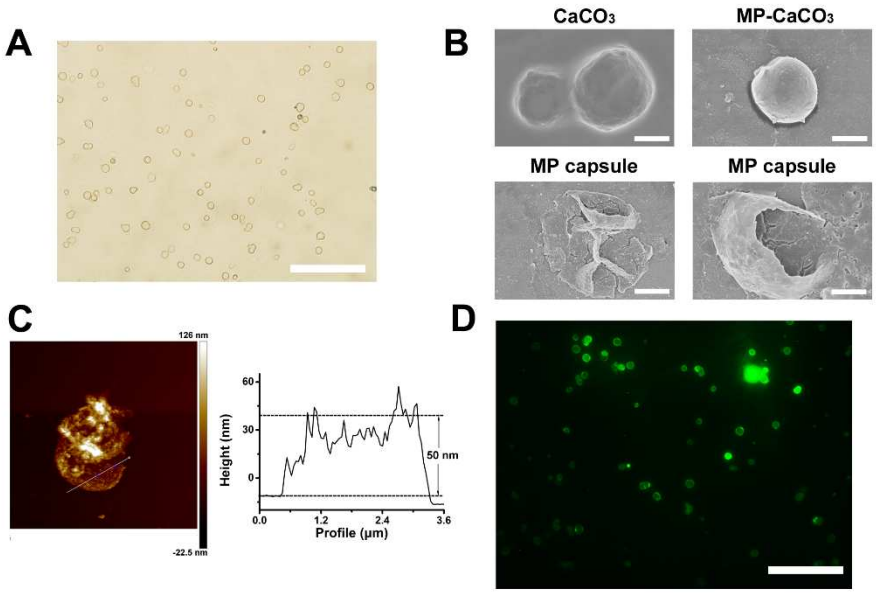


Figure 2. Preparation and characterization of the sMPC. (A) Optical microscopy image of prepared sMPC. Scale bar: 40 μm. (B) SEM images of prepared $CaCO_3$ particles and sMPCs. Scale bar: 1.5 μm (C) AFM image of prepared sMPC and its corresponding thickness profile. Note the height profile is the thickness of the two sides of sMPC. (D) Fluorescence microscopy images of the FITC-labeled antibody modified sMPC. Scale bar: 40 μm.

Soft MP capsules were prepared by mixing $PbCl_2$ and TA in the presence of PSS-stabilized calcium carbonate ($CaCO_3$) particle template. The strong coordination interaction between Pb^{2+} and TA results in the formation of metal-polyphenol network on $CaCO_3$ particles. After removing the template, sMPCs are obtained. Monodisperse, hollow, and round capsules were readily seen under optical microscopy (Figure 2A). The successful preparation of those capsules is due to the adsorption of the polyphenols and simultaneous coordination interaction between TA and Pb^{2+}. Adjacent hydroxyl groups of TA offer chelating sites for Pb^{2+} ions, and the plenty of gallol groups on TA promote efficient coordination-driven cross-linking, thus producing three dimensionally stabilized capsules. The number of sMPC in solution can be counted using flow cytometer, which could reach 3.5×10^8 particles/mL. Thus, the number of Pb^{2+} in each capsule was estimated to be 7.86×10^7 /particle according to inductively coupled plasma mass spectrometry (ICP-MS). Such a large metal content may be very beneficial to electrochemical detection compared with small metal particles that usually contain 10^4-10^5 metal ions. As shown in Figure 2B, scanning electron microscopy (SEM) images show that the prepared $CaCO_3$ templates (diameter ≈ 3 μm) were spherical and solid with a rough surface. After coating with MP, the $CaCO_3$ particles became smooth. The dried sMPC had features such as fractures, folds and creases, because of the collapse of thin membrane during the air-drying process. Atomic force microscopy (AFM) image further revealed the thickness of the prepared MP capsules was about 25 nm (Figure 2C), which was slightly thicker than that reported for MP capsules. The Young's

modulus value of the prepared capsules was around 56 KPa, which was 50-fold smaller than that of solid $CaCO_3$ (2.8 MPa).

The MP network displaced high affinity toward biomacromolecules,(Richardson et al. 2015) so the surface of prepared capsules could be easily modified with antibodies by simple one-step incubation. After conjugation of FITC-labeled anti-human IgA, strong green fluorescence was observed around the MP capsules, indicating the successful modification of antibodies on the capsules (Figure 2D). The interaction between antibodies and capsules was rather robust, and there was no obvious leaky of antibodies from capsules during a 9-day storage (Figure S3). Moreover, the synthesized sMPCs were sensitive to acidic environment, but had good stability in neutral pH (Figure S4), making them suitable for ASV analysis.

3.3 Comparison of sMPC and hMP-CP on electrochemical response

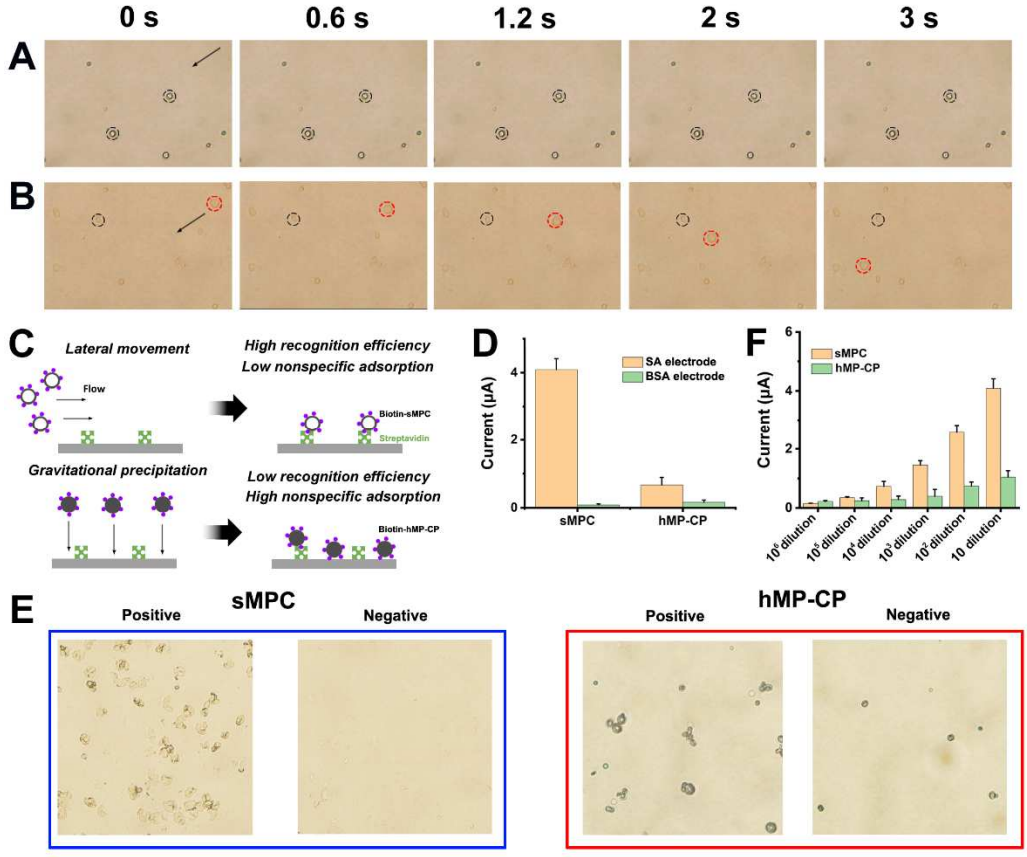


Figure 3. The surface behavior of (A) hMP-CPs and (B) sMPCs dropped on the electrode surfaces and their movement after injection of washing buffer. In Figure 3A, the black circles indicate the site of solid particles. In Figure 3B, the red circle indicates the movement of one sMPC and the black circle indicates an immobile particle as a reference. The black arrows indicate the flow direction. (C) The schematic illustration of the potential surface behavior of sMPCs and hMP-CPs on the streptavidin-modified surface and its effect on the molecular recognition. (D) Electrochemical responses of sMPC and hMP-CP on streptavidin (SA) or BSA-modified electrode surface. (E) Optical microscope images show the specific or nonspecific adsorption of the two particles on streptavidin-modified electrodes (positive) or

BSA-modified electrode surface (negative). (F) The ASV response of the two metal probes with different dilutions. Error bar represents three independent experiments.

After demonstrating the successful synthesis of MP capsules, we made use of a flowing electrochemical cell to monitor surface behavior of particles (Figure S5). Hard MP-coated $CaCO_3$ particle (hMP-CP) was employed as a control, and the only difference with sMPC was its solid core. After dropping hMP-CP on transparent indium tin oxide (ITO) electrode, the particles precipitated on the electrode surface within seconds, which was mainly due to gravitational sedimentation. Moreover, once sediment on the electrode surface, these hard particles (in the black circles) kept still on the electrode surface and barely underwent lateral motion even with a rapid flow rate (FR) of 300 μL/min (Figure 3A). Nevertheless, further increasing the flow rate of buffer, almost all of solid particles were suddenly washed away (data not shown). These surface behaviors made hard MP particles difficult to specifically recognize analytes on the surface because of the limited collision chance between particles and analytes. As a comparison, flexible, light-weight MP particles (in the red circle) slowly precipitated on the surface, and more importantly, they could freely roll over the solid surface with slight disturbance (Figure 3B). This surface behavior suggests soft MP particles have more chances to interact with their analytes and thus produce larger detection signals compared with hard MP particles. As such, the surface behavior of the two probes on electrode surface and the potential effect on recognition efficiency were outlined in Figure 3C.

Next, we evaluated recognition efficiency by using biotin-labeled BSA-modified probes and streptavidin (SA)-modified electrode. After incubation the electrode with the two probes, the strong interaction between SA and biotin allows the specific adsorption of particles on electrode surface. After rinsing, nitric acid (0.01 M) was added to destroy the probes and the released metal ions were detected by ASV. As shown in Figure 3D, the current signal produced by sMPC was 9.1-fold higher than that of hMP-CP. On the other hand, when the electrode surfaces were modified with a control protein, i.e., bovine serum albumin (BSA), the background signal of sMPC was also much lower than that of hMP-CP. Thus, the signal-to-noise ratio (SNR) of sMPC reached to 51.5, which was much larger than that of hMP-CP (SNR = 4.2), further suggesting the advantage of sMPC for electrochemical analysis. Figure 3E revealed the number of adsorbed sMPCs on the SA electrode was much more than that of hMP-CP, which indicated the enhanced signal mainly resulted from the better recognition efficiency of sMPC. As for BSA-modified electrode, most of the precipitated soft particles were easily washed away from the surface with only a single injection of washing buffer, implying the weak interaction between sMPCs and the underlying electrode. However, some hMP-CPs were still sticky to the surface due to their heavy weight, and large FR (700 μL/min) was needed to remove these particles. It was worth noting that such large FR also remove some specific adsorption particles, further compromising the positive signal. When the two probes were used as metal probe for ASV analysis, the analytical performance of sMPC was much better than hMP-CP. The robust ASV signal of sMPC could be detected even

under 10^6 dilution, corresponding to about 1000 soft particles in the solution, which was 10^3 times lower than that of hMP-CP (Figure 3F).

3.4 Electrochemical detection of EBVCA-IgA antibody

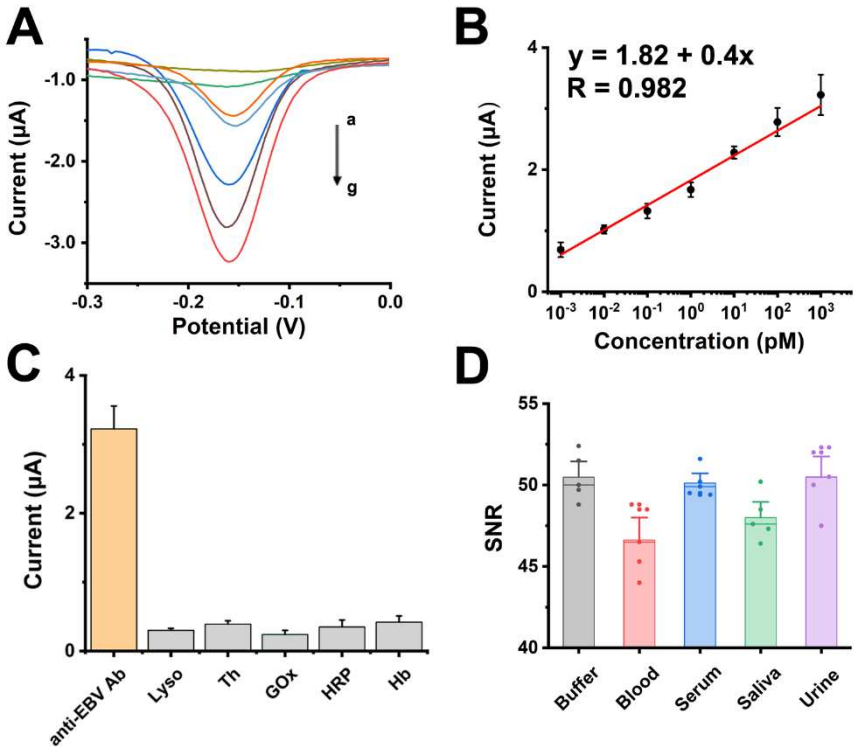


Figure 4. ASV profiles of the sMPC-based immunosensor incubating EBVCA-IgA with different concentrations of (a-g): 0.001, 0.01 0.1, 1, 10, 100, and 1000 pM. (B) Calibration plot of ASV response vs the concentration of EBVCA-IgA antibody. (C) Specificity of the electrochemical biosensor toward blank control and different control proteins: lysozyme (Lyso), thrombin (Th), glucose oxidase (GOx), horseradish peroxidase (HRP), and hemoglobin (Hb). The concentration of each control protein was 100 nM, which was 100-fold more than that of target antibody (1 nM). (D) The SNR of the immunoassay for detection of anti-EBV antibody spiked in different biological matrixes.

We made use of a classic sandwich structure to detect EBV antibody using EBVCA as antigen, BSA as blocking agent, and sMPC as signal generator. The protein modified electrode surface was characterized by electrochemical impedance spectroscopy (EIS), demonstrating the successful preparation of working electrode (Figure S6). EBVCA-IgA with a range of concentrations was assayed, and the electrochemical response was proportional to the antibody concentration from 1 fM to 1 nM with a regression equation expressed as I = 0.4x + 1.82, and the correlation coefficient was 0.988. The detection of limit was calculated to be 0.46 fM according to 3σ/S. As a comparison, we also used the traditional enzyme-linked immunoassay to detect the same target using TMB as colorimetric generator, which revealed that it could only sense 10 pM of EBVCA-IgA (Figure S7). It suggests a 10^4-fold enhancement in sensitivity for the antibody detection. Of note, if bare sMPC without modification

of anti-EBVCA-IgA antibodies was used, no electrochemical response was observed, indicating the low nonspecific adsorption of the particles on electrode surface (Figure S8). The proposed sMPC-based electrochemical method exhibited enhanced sensitivity and wider detection range compared with previously reported methods (Table 1), which was ascribed to the use of appropriate sensing probe.

Table 1. Comparison of different methods for virus antibody detection.

Methods	Strategies	Linear range	LOD	Ref.
Colorimetric	Viral Nanofibers	0-200 pg/mL	1.1 pg/mL	(Wang et al. 2015)
Fluorescent	Peptide-based FRET	0-300 nM	39 nM	(Golynskiy et al. 2010)
Fluorescent	DNA-based proximity extension assay	0.1 pM-10 nM	48 fM	(Lundberg et al. 2011)
Electrochemical	DNA-based amplification	0.05-6.4 ng/mL	0.7 pg/mL	(Song et al. 2014)
Electrochemical	Paper-based microfluidic platform	300-10^7 pg/ml-	300 pg/mL	(Zhao and Liu 2016)
Electrochemical	Antibody catalyzed water oxidation pathway	5 pM-500 nM	2 pM	(Welch et al. 2014)
Electrochemical	Metal-phenolic capsule	1 fM-1 nM	0.46 fM	Present work

The specificity of the proposed method was also investigated by using a series of control proteins such as lysozyme (Lyso), thrombin (Th), glucose oxidase (GOx), horseradish peroxidase (HRP), and hemoglobin (Hb). As shown in Figure 4C, no obvious ASV signals from control proteins were detected except that of analyte anti-EBV antibody (1 nM). In addition, immunoassay was also tested in different matrixes such as buffer, blood, serum, saliva, and urine (Figure 4D). Compared with buffer system, the ASV response in complex matrixes was almost unchanged (< 5.7% variation). These results revealed good specificity of this method for detection. The reproducibility of the method was also evaluated by inter-assay (one electrode was detected six times for 1 nM anti-EBV antibody) and intra-assay (six electrodes were tested with 1 nM anti-EBV antibody). The relative standard deviation (RSD) of inter-assay and intra-assay was 5.5% and 3.2%, respectively, demonstrating the acceptable prevision of this method.

3.5 The analysis of clinical samples

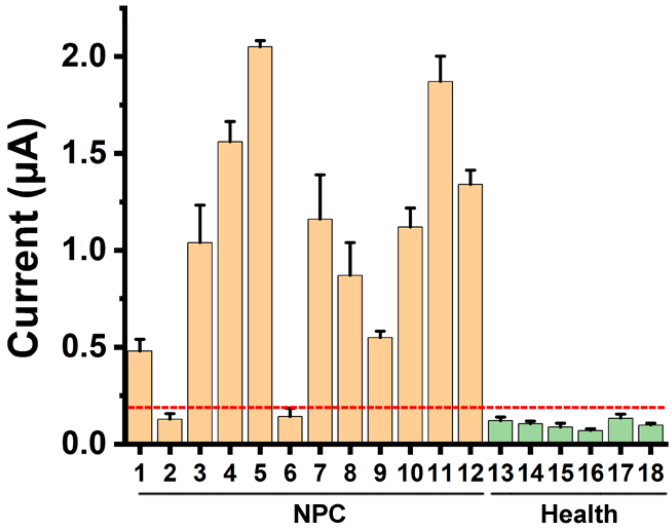


Figure 5. Detection of multiple clinical NPC samples by sMPC-based electrochemical assay. Error bars show the standard deviations of three independent measurements

To further demonstrate the utility of this sMPC-based electrochemical immunoassay in real clinical diagnosis, we used it to detect the EBVCA-IgA in the serum of NPC patients from local hospital. In clinical diagnosis, EBV-positive indicates that the detected signal is above the cutoff value, whereas that below the cutoff value reveals EBV-negative. After Ab-screening, 10 samples of 12 NPC patients, i.e., #1, #3, #4, #5, #7, #8, #9, #10, #11, and #12, have been identified as EBV-infected patients, demonstrating the high correlation between EBV infection and NPC (83.3%). The results were also in good agreement with the clinical reports (Table S1), further supporting the feasibility of the proposed method in clinical samples analysis.

4. Conclusion

In summary, we have proposed a sMPC-based electrochemical method for ultrasensitive detection of EBV. We demonstrate that sMPC are particularly suitable for ASV analysis because they leverage the molecular recognition and signal amplification. Solid, heavy-weight metal particles rapidly precipitate on the surface and hardly move on the electrode surface, which have less chance to interact with analytes, leading to reduced recognition efficiency. Nevertheless, soft, light-weight particles can freely move on the electrode surface, so they possess more chance to bind with corresponding analyte. Meantime, the micro-sized sMPC contains a large number of metal ions, which facilitates the signal amplification. Based on these, the sensitivity of the designed strategy has been greatly enhanced and allows the detection of sub-fetomolar analyte. Furthermore, the surface of sMPC can be easily modified with other recognition molecules such as DNA aptamer, peptides, or antibodies, so the method may be readily extended for the detection of other analytes, providing great potential for the detection of other biomarkers in clinical diagnosis.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi.

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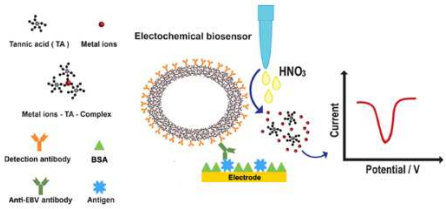
TOC



1. A metal-polyphenol capsule (MPC)-based electrochemical biosensor is proposed for detection of Epstein-Barr virus capsid antigen antibody (EBVCA-IgA).
2. The soft MPC can roll along the electrode surface which significantly improves its recognition capacity.
3. Taking advantage of the unique surface behavior of MPC, the proposed method shows an ultralow detection limit of 0.46 fM for EBVCA-IgA.
4. The method sensor allows efficient analysis of EBVCA-IgA in patients' samples.

Conflict of interest

The authors declared that they have no conflicts of interest to this work.