Supporting information

Preparation of A Novel Sandwich-Type Electrochemical Immunosensor for AFP Detection Based on ATRP and Click Chemistry Technique

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Experimental Section

2.1 Reagents

2-Hydroxyethyl methacrylate (HEMA, ≥ 99%, J&K SCIENTIFIC LTD) was passed through a column of Al₂O₃ to remove the inhibitor and stored at 4 °C. Acrylonitrile (AN, ≥ 98%, Tianjin FuChen Chemical Reagents, China) was distilled and stored at 4 °C. Ethyl 2-bromoisobutyrate (EBiB, ≥ 98%, Aladdin Chemistry Co., Ltd), copper bromide (CuBr₂, ≥ 98.5%, Tianjin DaMao Chemical Reagents Co., Ltd, China). 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA, ≥98%) was purchased from Beijing HWRK Chem. Anthraquinone-2-carboxylic acid (≥ 99.5%) was obtained from Zhengzhou Alfachem. Tetrahydrofuran (THF, ≥ 99.5%) and ascorbic acid (Vc, ≥ 99.7%) were brought from Tianjin Bodi Chemical Company. Bovine serum albumin (BSA) and 2-bromoisobutyryl bromide were purchased from Sigma-Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysuccinimide (EDC and NHS, ≥ 99.5%, Shanghai Yuanye Biological Technology Company, China), sodium azide (NaN₃, ≥ 98%, ChengDu Micxy Chemical CO., Ltd, China), ammonium chloride (NH₄Cl, ≥ 99.5%, Sinopharm Chemical Reagent Co., Ltd, China), diethylenetriamine (DETA, ≥ 99%, Sinopharm Chemical Reagent Co., Ltd, China) and 2-bromoisobutyryl bromide (≥ 98%, Aladdin Chemistry Co., Ltd) were all used as received. EDC/NHS, AFP, primary antibodies (Ab1) and secondary antibodies (Ab2) were all dissolved in phosphate buffered saline (10 mM, pH = 7.4). The above solutions were all prepared with ultra-pure water (18 MΩ cm). Real human serum was obtained from healthy human, which was a donation from Yuhuangding Hospital affiliated to Qingdao University. Blood samples were thoroughly centrifuged for 10 min, and the serum was stored in 4 °C for further use. Finally, the obtained human serum was further diluted for 100 times with 10 mM PBS.
2.2 Characterization

The number-average molecular weight \((M_n)\) and molecular weight distribution \((M_w/M_n)\) of the synthesized PHEMA and PAN were analyzed by Gel Permeation Chromatograph (GPC, Waters 1515) equipped with a refractive-index detector (Waters 2414) using HR column (7.8×300 mm). The Raman spectra of GO, GO@Br and GO@PAN were measured on Jobin Yvon Horiba spectrometer (France, Model T64000) at an excitation wavelength of \(\lambda_{\text{max}} = 514\) nm. Argon-Krypton mixed ion gas laser (MODEL 2018 RM, Make Spectra Physics, USA) was used as an excitation source. X-ray diffraction (XRD) patterns were collected using a Rigaku D/max 2500 VPC X-ray diffractometer. The FT-IR spectra measurements were performed on a Nicolet is 50 (Thermo Fisher Nicolet, United States). Fourier transform infrared spectrometer equipped with Thermo Nicolet corporation OMNIC 32 software. Energy dispersive spectrometer (EDS) was measured on Bruker XFlash 6160 under a nitrogen atmosphere. The CHI 660C electrochemical workstation (CH Instruments, Shanghai) with traditional three-electrode system was used to carried out the square wave voltammetry (SWV).
Figure S1. The visible calibration curve of standard anti-AFP.
SEM has been used to characterize the formation of sandwich-type electrochemical immunosensor. The assembly process is roughly divided into four parts, the morphology of GCE have been characterized as observed in Fig. S2. The rGO/AuNPs was coated on the surface by electrochemical deposition. From the Fig. S2a, some gold nanoparticles and graphene oxide were successfully modified to the GCE. After the antibody Ab1 was added to the surface, the GCE was covered with a layer of large protein molecules in Fig. S2b. The antigen was then added and combined with the Ab1, so the particle size of the molecules was increased as shown in Fig. S2c. Finally, Fig. S2d clearly showed that the nanoprobe was successfully loaded on the surface of GCE. The whole sandwich-type electrochemical immunosensor was proved to successfully assemble.

Figure S2. The SEM images of (a) rGO/AuNPs/GCE, (b) Ab1/rGO/AuNPs/GCE, (c) antigen/Ab1/rGO/AuNPs/GCE, (d) nanoprobe/antigen/Ab1/rGO/AuNPs/GCE.
Figure S3. (a) Pseudo-first-order kinetics investigation of PAN initiated by EBiB in presence of GO@Br; (b) Number-average molecular weight ($M_n$) and molecular weight distribution ($M_w/M_n$) vs monomer conversion for the SI-ATRP of PAN; (c) Pseudo-first-order kinetics investigation of PHEMA. (d) Number-average molecular weight ($M_n$) and molecular weight distribution ($M_w/M_n$) vs monomer conversion for the ATRP of PHEMA.
Table S1. Contributions of individual chemical moieties in the high-resolution C1s spectra of GO and GO composites.

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<thead>
<tr>
<th>Samples</th>
<th>XPS (atom%)</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>GO</td>
<td>62.25</td>
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<tr>
<td>PAN-g-GO</td>
<td>57.53</td>
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<tr>
<td>P(VT-co-HEMA)-g-GO</td>
<td>48.95</td>
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