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# Layer-by-layer assembly of hemoglobin and gold nanoparticles for enhancing the ability of oxygen carrying

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ABSTRACT

This paper reports layer-by-layer films fabricated with hemoglobin (Hb) and gold nanoparticles (AuNPs) as well as their applications in carrying oxygen. The characterization of $\{Hb/AuNPs\}_n$ multilayer films at different layers revealed that the formation of films was step-by-step. Meanwhile, the study showed that the $\{Hb/AuNPs\}_n$ modified glass carbon electrode can maintain the biological activity of Hb. The capacity of carrying oxygen was associated with the number of assembled layers. The $\{Hb/AuNPs\}_3$ multilayer films displayed a good storing and releasing ability of oxygen. The study can provide an alternative method for construction of hemoglobin function interface with high-efficiency oxygen carrying capacity.


## 1. Introduction

Traumatic injury is often accompanied with hemorrhagic shock, and blood transfusion is needed in most emergency situations. However, the donor blood used in medical practice has several limitations, so the study of artificial red blood cells has become an important research field [1]. In this climate, the construction of artificial red blood cells with high-efficient oxygen carrying capacity is highly meaningful for satisfaction of patients with postoperative hypoxic-ischemia, cardiorespiratory failure, and physiological requirements of special people in aeronautics as well as deep sea diving [2,3]. Red blood cells (RBCs) are one of the most cells in blood, which ensure the oxygen supply to human organs and tissue through transportation of oxygen and carbon dioxide [4]. Hemoglobin (Hb), the vital component of RBCs, plays the important role in carrying oxygen [5]. Thus, it is very importance to seek safe and high oxygen-carrying blood substitutes based on Hb [6–9]. Among them, the construction of Hb bionic function interface with high-efficiency oxygen carrying capacity has become a bottleneck.

The technology of layer-by-layer assembly is used to develop multilayer films based on electrostatic incorporation. It has a significant impact on many fields including cellular and tissue engineering, and protein multilayer architectures [10–12]. Layer-by-layer assembly of Hb with the various nanoparticles, such as silver (Ag), multiwall carbon nanotubes and $Fe_3O_4$@Pt for the pH-switchable behavior or biosensors have been reported in our previous study [13–15]. Gold nanoparticles (AuNPs) were widely used in biosensors and biomedicine because of their good biocompatibility, high surface area, special catalytic activity, and the convenience of controlled fabrication [16–18]. The AuNPs can provide more binding sites for the coupling of biomolecules (e.g. proteins and enzymes) without losing their biological activity [19,20]. Our previous studies found that the Hb immobilization on AuNPs modified electrode retained its biological activity [21,22].

In this study, the $\{Hb/AuNPs\}_n$ function interface was constructed through nanotechnology and self-assembly techniques. The oxygen carrying capacity of $\{Hb/AuNPs\}_n$ function interface was studied through electrochemical methods. The results showed that $\{Hb/AuNPs\}_3$ function interface showed a high-efficiency

*Abbreviations:* AuNPs, gold nanoparticles; Hb, hemoglobin; RBCs, red blood cells; CV, cyclic voltammetry; DPV, differential pulse voltammetry; GCE, glassy carbon electrode; SCE, saturated calomel electrode; SEM, scanning electron microscopy; TEM, transmission electron microscope; EIS, electrochemical impedance spectroscopy; PBS, phosphate buffered saline.

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## 2. Experimental

### 2.1. Reagents and materials

Bovine hemoglobin was purchased from Sigma–Aldrich (Shanghai, China). Chitosan (92.5% deacetylation, CS) was purchased from Nantong Shuanglin Company. $HAuCl_4{\cdot}4H_2O$ (Au% > 48%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Potassium ferricyanide ($K_3Fe(CN)_6$) and potassium ferrocyanide ($K_4Fe(CN)_6$) were purchased from Shanghai Chemical Plant (Shanghai, China). Phosphate buffered saline (PBS) was prepared by mixing $Na_2HPO_4$ and $NaH_2PO_4$ and adjusting the pH value to 7.0 with 0.10 M $H_3PO_4$ or NaOH solutions. All solutions were prepared with twice-distilled water and stored at 4 °C.

### 2.2. Apparatus and measurements

All the electrochemical measurements were performed on a CHI660D electrochemical workstation (Shanghai Chenhua Instrument Company, China), which was equipped with three-electrode system. The glassy carbon electrode (GCE) (Ø = 3 mm) was used as a working electrode, a platinum wire was used as a counter electrode and a saturated calomel electrode (SCE) was used as a reference electrode. The solution was purged with high-purity nitrogen for at least 15 min before electrochemical measurements.

The morphology of AuNPs was studied by FEI Tecnai-12 transmission electron microscope (TEM). Scanning electron microscopy (SEM) images were obtained with HITACHI S4800 SEM (Hitachi, Ltd., Tokyo, Japan). The electrochemical impedance spectroscopy (EIS) performed with AUTOLAB PGSTAT 302N electrochemical working station (Metrohm Co. Ltd., Switzerland). The UV–vis spectra of the samples were recorded using a UV-2450 spectrophotometer (Shimadzu, Japan).

### 2.3. Construction of the $\{Hb/AuNPs\}_n$ multilayer films

AuNPs were prepared by adding $Na_3$ citrate solution to a boiling $HAuCl_4$ aqueous solution according to the literature [17]. Bare glassy carbon electrode was firstly polished with abrasive paper and then with alumina slurry (1.0, 0.30 and 0.05 μm), followed by ultrasonically cleaned in ethanol and water. As shown in Fig. 1A, the chitosan thin film was prepared on the polished glassy carbon electrode surface by electrode position in 2.0 mg/mL chitosan solution

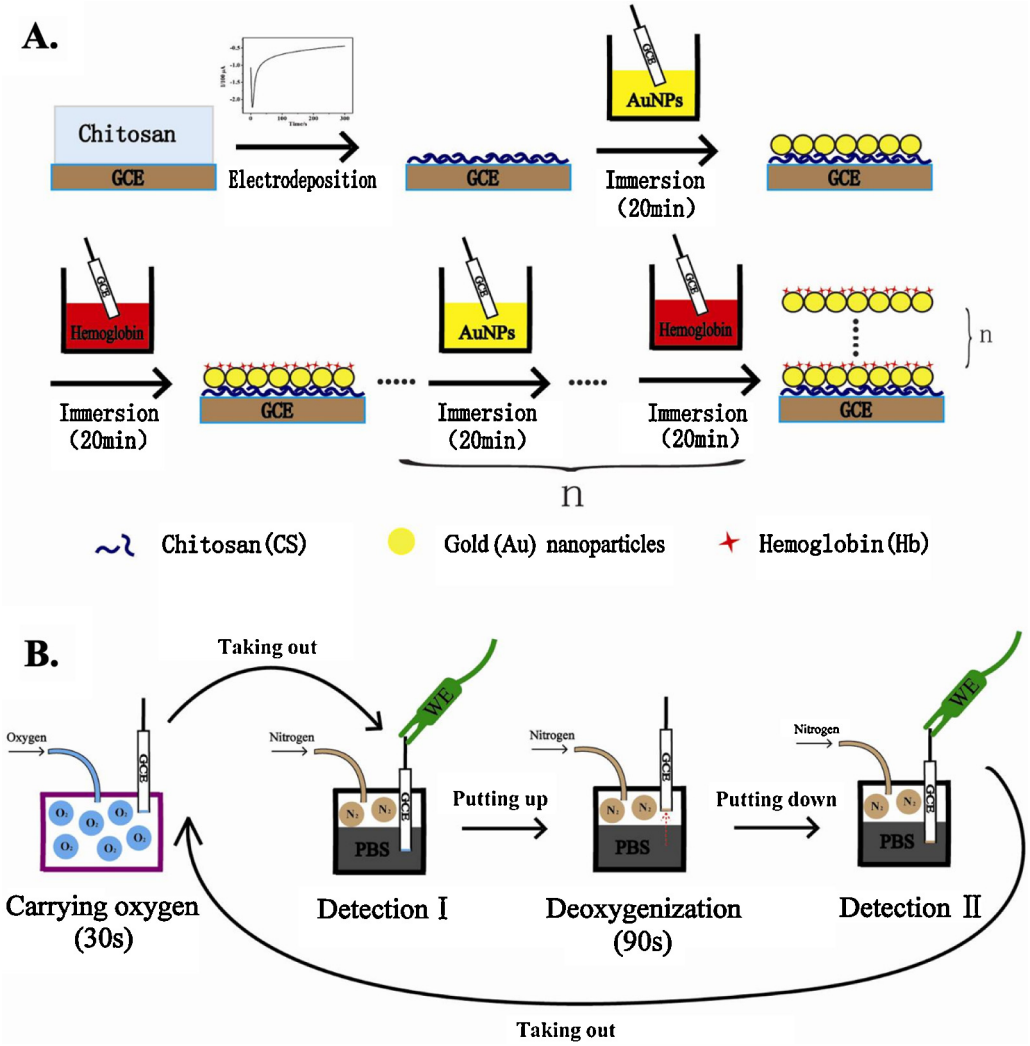


**Fig. 1.** (A) The assembly process of the $\{Hb/AuNPs\}_n$/CS/GCE. (B) The technological process for studying oxygen-carrying ability of $\{Hb/AuNPs\}_n$/CS/GCE.