A novel membrane with heterogeneously functionalized nanocrystal layers performing blood separation and sensing synchronously

A novel sensing membrane with heterogeneously functionalized nanocrystal layers can simultaneously perform in-situ blood separation for the serum extraction and electrochemical sensing of various physiological indexes.

A novel membrane with heterogeneously functionalized nanocrystal layers was designed to synchronously perform in situ blood separation and sensing for the extraction of pure serum without any blood cells and fibrinogen by size sieving, and simultaneously realizing the electrochemical analysis of various physiological indexes.

Biochemical testing of blood plays a critical role in clinical diagnosis, DNA sequencing, drug screening, cell culture, etc.¹⁻⁴ Efficient and accurate sensing of physiological substances is always a challenging task because of the complicated components in the blood system.⁵ Moreover, blood coagulation and other interference in a blood bioassay would make the analysis results highly sensitive to detecting environments, such as whole blood and serum. Therefore, effective target separation from the freshly collected blood before the analysis becomes crucial for ensuring the accuracy of the detection.⁶ Serum, plasma, blood cells and other components are then separated according to their different purposes with respect to the detecting targets. Currently, the separation and analysis processes are usually independently operated with specific techniques and instruments,⁷,⁸ resulting in a tedious procedure from sample collection to component analysis. In an emergency, a delay in obtaining the blood index might increase the rescue risk. To date, a variety of strategies for bio-extraction (e.g., centrifugal separation, membrane separation, and chemical extraction) and bio-analysis (e.g., biosensor detection, capillary electrophoresis analysis, and fluorescence microscopy mapping) have been presented⁹⁻¹⁴ to improve separation or analysis performance respectively. Synchronous technology and devices have not yet been explored well. Novel material facilitated in situ separation and sensing on a synchronous device would be able to bring about profound benefits to bioscience and clinical medicine. Herein, for the first time we proposed a novel sensing membrane by the construction of heterogeneously functionalized nanocrystal layers to achieve simultaneous membrane separation and electrochemical sensing.

In order to couple the technologies of blood separation and analysis, the desired membrane is required to possess the features of both a filter and an electrode. As shown in Fig. 1, with only one single electroactive material, the membrane channel was designed to possess two different functionalized layers (separation layer and sensing layer). The separation layer requires nanopores arising from the tight stack of irregular nanoparticles to produce the sieving channel. The channel is narrower than all sizes of blood cells and fibrinogen, and will only allow the entry of serum into the inner channel for achieving blood separation. To analyse the physiological index in situ, the sensing layer is fabricated by regular nanocrystals with a high electrocatalytic activity to induce an electrochemical oxidation with targets. When working, a weak external potential is imposed across both layers for the excitation of electrocatalytic function of the sensing layer. Then redox electrons will be generated and transferred through the same channel of serum access to produce the response current for blood sensing. According to the above design, not only can pure serum be in situ extracted during
the collection of fresh blood, but also instant blood assay can be achieved.

To prove the above concept, we used a well-known electrocatalytic material, Prussian blue (PB), as the sample membrane material. Its regular nanostructure has been confirmed to possess much higher electrocatalytic activity than the one of an irregular morphology. The sensing membrane was prepared by a vacuum driven self-assembly method (Fig. S2, ESI†) on a support of porous Al₂O₃ hollow fiber which was built with the architecture of a connected sponge pore (SP) and finger pore (FP) for inducing the growth of a heterologous PB nano-morphology. As seen in the cross-sectional view of the PB membrane in Fig. 2a, the intergrowth of PB irregular nanoparticles (Fig. 2b and c) constructed the nanopores on the SP layer to establish separation channels. On the other hand, the FP layer can accommodate well-defined nanocubes (Fig. 2d and e) of PB for the good electrocatalysis of blood targets. This membrane architecture provides a “separation before sensing” feature that maintains the sensing accuracy, preventing interference from cells and bacteria in blood. The growth mechanism of PB heterologous nanostructures was investigated by cyclic voltammetry (CV) and EDS techniques (Fig. S4, ESI†). The high surface area of the SP layer allowed the fast formation of irregular crystals due to the limits of narrow space and high reactant concentration, and tended to grow numerous disordered crystal nuclei (Fig. S5, ESI†). Differently, in the FP layer, the cavities were wider to provide enough space for the free growth of PB crystals. Therefore, a sensing layer for electrocatalysis was generated by the uniform 40 nm PB cubes. Besides, a portable device (Fig. 2g) was also designed to install the as-prepared sensing membrane (Fig. 2f).

The separation performance of the sensing membrane was investigated in pure water and glucan systems. The porosity of the membrane was tested and was found to be ca. 28.9%.

![Fig. 2](image)

(a) Cross-sectional view of the PB membrane prepared by 40 self-assembled layers. (b–e) show the four FESEM images of the membrane outside surface, sponge porous (SP) layer, finger porous (FP) layer and inside surface structures. The bar in the inset is 50 nm. (f) Photo of the bare Al₂O₃ support and the as-prepared PB membrane made by a vacuum self-assembly approach. (g) Photo of the device composed of three electrodes in the sensing system and a hollow fiber separation module.

Under 0.09 MPa vacuum pressure, the flux kept stable for 70 min in the pure water and the average flux value reached 67.8 L m⁻² h⁻¹ (Fig. 3a). In order to match the size of fibrinogen (340 000 Da) which is the smallest among the separating targets for serum extraction, the molecular weight cut-off (MWCO) test of the membranes prepared by different self-assembled layers was conducted. As shown in Fig. 3b, the MWCO of the sensing membrane with 40 self-assembled layers was 253 000 Da which was much smaller than the molecular weight of fibrinogen. Thus, the constructed separation layer of the sensing membrane can be used well for rejecting fibrinogen by its appropriate pore size. The above results also demonstrated that the membrane pore size can be controlled by the number of self-assembly layers.

Does the membrane also have the electrochemical ability for sensing or not? We tested the conductivity and electrocatalysis of the membrane channel using the scanning vibrating electrode technique (SVET) and CV characterization, respectively. Compared with Fig. 4a and b, the current density distribution of the whole surface continuously changed. As shown in the selected areas 1 and 2 which respectively belonged to the sensing and separation layers in the two SVET maps, both regions produced a current flow. The current density of region 1 increased from cathodic 5.76 to anodic 0.49 μA cm⁻², while the density of region 2 decreased from anodic 3.27 to cathodic 0.12 μA cm⁻². The results indicate that the response current can be rapidly transmitted through the electrolyte-immersed PB membrane with low resistance (Fig. S6, ESI†). Glucose was used as the target to evaluate the biosensing performance of the PB membrane. Silane modified graphene oxide (GO) was used as an enzyme carrier to bridge the enzyme to the Al₂O₃ surface (Fig. 4c and d).

Because of the blocking caused by the tight separation layer, the enzyme can only localize to the sensing layer without covering the outer surface (Fig. 4e). This distribution can be beneficial to protect the enzyme from direct contact with blood which contains various bacteria that can affect the accuracy of detection. Meanwhile, the rare enzyme existing in the separation layer can weaken the electrocatalysis of this layer. When the serum is separated from the blood, the serum glucose can rapidly produce H₂O₂ by enzyme oxidation in the sensing layer. Through the serum transportation, H₂O₂ can be effectively electrocatalysed to generate a current response by the PB nanocubes. During the
Fig. 4 (a and b) Online current density maps of the cross-section of the PB membrane by SVET scanning. The maps were recorded at the beginning and after 5 min of immersion in 0.1 M KCl solution. (c) Schematic of the sensing function generation by the functionalization of graphene oxide (GO) as the enzyme carrier. (d) FT-IR spectrum for characterization of pure GO, silane activated GO, and GOD loaded GO. (e) Photographs of the GOD/silane/GO solution before and after the vacuum channel deposition. (f) CV reduction segment of the PB membrane installed device for the detection of glucose solutions of 0.1, 0.2, 0.5, 1.2, 4, 6, 8, 10, 12, 14, and 16 mM. (g) Linear calibration of reduction current peak value vs. glucose concentration.

detection of glucose, increasing concentrations were correlated with increased reduction in the peak currents (Fig. 4f), showing a detection sensitivity of 16.06 ± 1.41 μA mM⁻¹ for glucose in the linear range from 0.5 to 16 mM (Fig. 4g). The PB membrane can also be used to test lactate and glutamate levels with excellent sensitivity and linear range by changing the different enzymes (Fig. S8, ESI†).

Finally, the online “synchronous separation and sensing” performance of the as-prepared sensing membrane was evaluated after installation in the portable device (Movie S1, ESI†). Because of the special design of the electrode micro-architecture, the transfer directions of the electric current and serum were opposite to provide an adequate enzyme reaction time although the same physical channel was used for both sensing and separating processes. Fresh whole blood from mature rabbits was collected as the sample for processing. The synchronous performance of blood separation and sensing is shown in Fig. 5a and b. Within 30 s, the separated serum had not filled all the channels to reach the inner tube; therefore, the electrocatalytic electron transfer was hindered and the current loop remained turned off. When the serum was initially collected in the inner tube, the signal channel was switched on, and the electrons were transferred by the serum electrolytes. After that, a stable current was maintained with the

separation in progress, which assured the accuracy of analytical results. At approximately 60 s, CV scanning revealed that the blood sugar index detected by this device was 9.97 mM (Fig. 5c), very close to the 10.2 mM measured by the commercial glucometer. Subsequently, the collected light yellow serum sample was further analysed (Fig. 5d). As shown in Fig. 5e and f, erythrocytes, leukocytes and platelets were completely rejected, and the serum production rate can reach 0.27 mL min⁻¹. In addition, because of the hydrophilic membrane surface, viscous blood cannot accumulate significantly on the surface of the membrane, preventing a sudden decrease of flux (Fig. S9 and S10, ESI†). Besides, the device was kept rotating up and down to force the strong movement of blood during working, which can avoid well the adhesion of blood cells from the blood coagulation.

The above bifunctional synchronization relies on the excellent stability of the membrane. The adhesion stability of the PB membrane was tested by the nano-indentation technique.24,25 The failure point of scratch occurred at a 20.92 mN loading force with a 3.58 μm membrane thickness (Fig. S11, ESI†). Membrane tearing began as a slight imprint and progressed to a deep scratch; however, no peeling occurred, reflecting the great mechanical strength between PB and the Al₂O₃ support. In addition, after 50 repeats of the CV scanning, the redox peaks did not show an obvious decrease, which confirmed the electrochemical stability of the PB membrane (Fig. S12, ESI†). The simulated results from density functional theory (DFT) showed that the electron cloud of Al happened to overlap with Fe, C, and N which revealed the possibility of their binding behaviors (Fig. S13, ESI†). XPS further confirmed that peaks of Fe²⁺ produced a slight shift to high binding energy which indicated the loss of electrons. Moreover,
C and N contributing to the C-N bond both produced an obvious low energy shift; especially, a new energy peak arising from the Al-N bond appeared in the N1s spectrum (Fig. S14, ESI†). According to the combination of theory simulation and XPS experiments, the stability of the PB membrane is mainly attributed to the chemical interaction between the CN group from PB and the Al element from the Al2O3 support. Due to the strong stability, the membrane can maintain 97.8% sensitivity with the same separation performance after 10 days of storage.

In summary, we designed a novel sensing membrane for achieving a synchronous blood separation and sensing platform. The as-prepared membrane can efficiently collect high quality serum, and instantly detect blood indexes from whole blood via bifunctional channels for the simultaneous transfer of serum and electron. Not limited to PB, more and different electrocatalytic materials can be applied in the fabrication of the sensing membrane. This sensing membrane concept provides a new strategy for the acceleration of blood extraction and analysis, as well as promising application in the urgent blood assay for clinical diagnosis.

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Notes and references