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A novel separation–sensing membrane performing precise real-time serum analysis during blood drawing

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Abstract: Dynamic and on-site analysis of serum from human blood is crucial especially for making decisions during clinical surgery and emergency treatments. However, state-of-the-art blood assay methods can only collect single or discrete data of physiological analytes; thus, remaining a great challenge in the online reports of the dynamic fluctuation of key analytes. Here, we propose a novel separation-sensing membrane by constructing a heterogeneous-nanostructured architecture, wherein a surface nanoporous layer continuously extracts serum, while the biosensing nanochannels underneath dynamically recognise biotargets, thereby achieving a continuous testing of vital clinical indices as blood is drawn. By precisely controlling the pore structure and nanoshape of biosensing crystals, this membrane achieved accurate and online glucose and lactate monitoring in patients with a variety of medical conditions within 1 min, which is one order of magnitude faster than the state-of-the-art techniques. Moreover, various kinds of bio-recognition sensors can be introduced into this membrane to accurately detect glutamate, transaminase, and cancer biomarkers. This separation-sensing membrane provides a versatile platform for online and dynamic clinical diagnosis.

Introduction

Blood analysis is a keystone for diagnosing and evaluating diseases[1]. However, current blood assay technologies are inadequate in terms of both accuracy and timeliness, even in countries with advanced medical systems. In the United States, approximately 12 million outpatients are subject to diagnostic errors each year[2]. Moreover, 21% of trauma deaths might have been avoided if immediate diagnosis was performed[3]. Real-time monitoring of vital blood indicators, such as lactate and glucose, is crucial for treating critical illnesses[4]. For example, a 20% reduction in the relative risk of all-cause mortality in patients with sepsis is achievable by the timely assessment of lactate levels[5]. Especially for surgery or emergency, not only is a fast report of key physiological indices required but also is an online and dynamic monitoring method to indicate the fluctuation of analytes desired, which is essential to guide the operation. Presently, real-time and dynamic analyses remain difficult with conventional blood assay methods (e.g., biochemical analyser for transaminase, immunoassay analyser for cancer biomarkers, and quantitative polymerase chain reaction for viruses)[6] because serum must be extracted from blood via an independent procedure prior to biochemical analysis[7]. For example, immunoassays cannot use whole blood directly because the reagents can damage cells and interfere with the actual serum composition[8]. Hence, in hospitals, centrifugal separation is currently being clinically used for serum extraction, although this method is time-consuming (at least 20 min) and easily damages cells, thereby causing haemolysis to affect the real levels of blood components. Such independent separation approaches also cause a delay in the assay results of the early diagnosis of short-course diseases, such as virus infection or cerebral infarction.

Some commercial devices, such as glucometer and biochemistry analysers can perform whole blood analysis without separation[9]. However, the glucometer detects glucose with a paper-type sensor, which is a one-time-use approach that can only report single data. Likewise, a biochemical analyser requires a pipette to draw the blood from a sampling tube into an electrolyte cell for analysis; an operation that is discontinuous and, thus, limited to reporting single data. Some advanced commercial devices (e.g., iSTAT[10]) contain a microfluidic device for blood separation based on the differences in the diffusion rate between serum and other blood components. In these devices, the fast coagulation of whole blood easily blocks the channel; thus, the microfluidic cartridge can only be used once, thereby making the devices to mainly serve as discrete data collection instruments. Overall, although these commercial devices can detect whole blood or have a blood separation unit, they face challenges in online and real-time whole blood analyses, which is urgently demanded for surgery and/or emergency scenarios. A prerequisite to achieve real-time serum analysis is in situ serum separation from whole blood during drawing. Membrane separation technology has shown the feasibility of continuous blood purification (e.g., dialysis[11]), wherein the toxins in the blood are removed by the membrane. Polymers, such as cellulose acetate, polysulphone, and polyethersulphone[12], are used to fabricate artificial kidneys for
blood purification, but are functionless in blood analysis because of the absence of a bio-recognition ability.

Therefore, we propose a separation-sensing (SepSen) membrane herein that possesses both separation and electrocatalytic functions to in situ extract serum and simultaneously recognise biomolecules of interest online during blood drawing (Fig. 1a). The SepSen membrane features heterogeneous nanostructures consisting of a porous surface separation layer over biosensor channels by coupling the coordination of Prussian blue (PB) with pyrrole (Py) polymerisation. The separation layer consists of continuous nanopores with a lower size than all the blood cells and fibrinogen (FIB) allows only serum to pass through without damage, while the biosensing channels are composed of regular-shaped nanomaterials and specific bio-recognitioners (such as enzymes, DNA, and antibodies), which can produce and magnify the detection signal to the objective serum biomolecules. For real-time serum analysis, which is essential to efficiently guide surgery, a portable device was built by assembling a SepSen membrane (serving as the working electrode, WE) with a counter electrode (CE) and reference electrode (RE) in a blood collection tube. As human blood is drawn into the tube under vacuum conditions, it is separated via the nanopores of the membrane surface layer. All serum components are smaller than the pores and can permeate through the membrane surface layer and pass into the biosensor underneath. The signals generated by the serum–bio-recognizer–analyte recognition device were collected by an electrochemical workstation connected to the three electrodes (i.e., WE, CE, and RE). A fast and smooth signal transfer circuit is formed with the flow of blood, thereby enabling dynamic monitoring of the vital indices in the blood.

Results and Discussion

Construction of heterogeneous nano-architecture in the SepSen membrane

Owing to the large differences in the principles behind membrane separation and electrochemical biosensing, it is difficult to integrate them into one membrane. Regarding material design, we used PB and polypyrrole (PPy) to construct a nanocomposite membrane with a functional balance between electrocatalysis and membrane formation. PB possesses high electrochemical activity for enzyme reaction but exhibits a weak construction ability of the porous structure, while PPy can easily produce a uniform and thin porous membrane during its polymerisation process but lacks catalytic capabilities. Therefore, the integration of these two materials as the SepSen membrane...
supplies the basic requirement for realising separation and biosensing functions. Additionally, we aimed to create a heterogeneous nanostructure for blood separation and biosensing channels for signal detection. However, routine methods for the synthesis of these two materials are quite different and easily produce a homogeneous membrane nanostructure[13].

To obtain the desired heterogeneous nanostructure of the SepSen membrane, we controlled the reaction kinetics of PPy and PB. Here, we coupled the polymerisation and coordination reactions as follows:

\[ \text{H} \text{N} + \text{Fe}^{3+} \rightarrow \text{H} \text{N}_n + \text{Fe}^{2+} \]  
\[ 3\text{Fe}^{2+} + 2[\text{Fe(CN)}_6]^{3-} \rightarrow \text{Fe}_2[\text{Fe(CN)}_6]_2^{3-} \]

In these coupled reactions, the Fe\(^{3+}\) ion serves as an oxidant for PPy polymerisation and is reduced to Fe\(^{2+}\); thus, becoming a reactant for PB formation. In this study, a precursor solution of a ferric and ferricyanide mixture was initially used to fill a homemade ceramic hollow fibre (Fig. 1b). Subsequently, a PPy solution was allowed to diffuse into the pores of the hollow fibre to initiate its polymerisation by oxidation with Fe\(^{3+}\) to produce Fe\(^{2+}\) that can coordinate with [Fe(CN)\(_6\)]\(^{3-}\). Therefore, PB formation occurs after PPy synthesis, leading to unequal reaction rates that favour the formation of the heterogeneous nanostructure of the resulting membrane. Therefore, the prepared membrane exhibited two different nanostructures on the surface and in the inner pores (Fig. 1c). At the top, a continuous layer with a thickness of 300 nm was created with homogeneous pores measuring approximately 50 nm, providing a channel for cell separation. Under this layer, the pore walls show different features and are composed of 50 nm nanocubic PB crystals with a polymer layer (Fig. 1c2). By comparing the distribution of iron and carbon at locations without regular crystals (Fig. 1d), we found that iron was rarely present, but the carbon signal was widespread, which confirmed the presence of PPy in the accumulation gap of the PB crystals. The different nanostructures of these two layers could be attributed to the concentration gradient of PPy from the outer to the inner support during membrane preparation. As it diffuses, the high concentration of PPy at its interface with the substrate surface accelerates the polymerisation reaction upon meeting Fe\(^{3+}\) ions (Fig. S1a to c). Thus, PPy could quickly form a polymer layer on the surface in the early part of the synthesis. Afterwards, owing to Fe\(^{2+}\) and [Fe(CN)\(_6\)]\(^{3-}\) filling the support pores, the PPy concentration gradually decreased along the channel direction, causing a sudden decrease in the rate of PPy formation and producing fewer Fe\(^{2+}\) ions. As the reaction progressed, the appearance of PB crystals in the pores gradually changed from an irregular shape to a well-defined nanocube (Fig. S1d–f); this mainly relied on the slow crystallisation rate due to the low Fe\(^{2+}\) concentration. Consequently, we obtained the outer irregular and inner regular nanocomposites at the same time to construct a separation–biosensing channel.

Figure 2. Heterogeneous nanostructure construction of SepSen membrane. (a) Dynamic mass monitoring of the membrane throughout the entire preparation process using a quartz crystal microbalance. Green dashed lines indicate the different growth stages. (b) Online UV-vis spectroscopy for synchronously monitoring the absorbance bands of PPy and PB. (c) Electrochemical coverage amounts of PB and PPy in the membrane prepared at 10, 20, and 60 min. (d) XPS patterns of the surface of the SepSen membrane synthesised for 10, 20, and 60 min. (e) Surface hydrophilicity of the membrane in water and serum. (f) Zeta potential measurements and calculated charge density of the membrane surface. (g) Continuous imaging of RBC movement as the membrane works at 0.1 MPa pressure.
To distribute the contribution of PB and PPy to the membrane formation, dynamic mass measurements (Fig. 2a) were applied to demonstrate that we could obtain three main growth stages (rapid elevation before 10 min, smooth increase from 10–20 min, and stable state after 20 min) from the preparation process. Online monitoring of the ultraviolet-visible (UV-vis) adsorption at approximately 320 nm and 733 nm, peaks of the bands derived from the π→π* electronic transition in PPy and the vibration of the CN groups in PB, respectively, enabled the distinct growth of PPy and PB\(^{14}\) in these three stages to be observed (Fig. 3c).

Initially, the PPy peak rapidly increased to a high intensity and then became steady. However, PB only exhibited a slow enhancement of its characteristic peak. At this stage, the ratio of the coverage by mass of PB to PPy was calculated to be approximately 0.42 (Fig. 2c) using the Faraday equation\(^{15}\) as follows to indicate that PPy is predominant in the membrane initially formed:

\[
\Gamma_T = \frac{Q}{nF}
\]

Where, \(Q\), \(n\), and \(F\) represent the total electric quantity of the single redox peak, the average number of electrons transferred, and the Faraday constant, respectively. The parameter \(Q\) was derived from the integral of the typical oxidation peak in the cyclic voltammogram (Fig. S2). During electrochemical redox scanning, the reduction or oxidation peak produced by electron transfer indicates the amount of material. At 20 min after the start of the reaction, the PB intensity increased more rapidly, whereas the PPy intensity remained relatively constant. At 60 min, the coverage ratio increased to 0.46, indicating an increase in the PB growth rate over time. The above-stated evidence confirms our expectation that the two coupled reactions enable the achievement of different PPy and PB growth rates.

The formation of a porous structure on the membrane surface is essential to provide continuous whole blood separation for always allowing fresh serum for dynamic detection. To achieve precise control of the pore structure, the separation layer was further studied to clarify its formation mechanism. Of the chemical species involved in the membrane synthesis, only the cyano ligands of \(K_3[Fe(CN)_6]\) and the PB product contained CN bonds; however, this form of carbon was not evident in the C1s X-ray diffraction peaks (Fig. 2d). This indicates a low rate of PB formation in the separation layer. The intense peaks at 397.8, 399.9, and 402.7 eV are typical of the −NH−, −N+H−, and =N+−, respectively, of PPy\(^{16}\). These peaks were observed over the entire preparation period; moreover, their intensities continued to increase from 10–60 min. This phenomenon is slightly different from that of Fe in PB. In the Fe 2p spectrum, the binding energies at 712.6 eV and 725.9 eV correspond to Fe 2p3/2 and Fe 2p1/2\(^{17}\), respectively, which arise because of the presence of Fe\(^{3+}\). Furthermore, the peaks located at 708.5 eV and 721.4 eV are assignable to Fe\(^{3+}\) in the \([Fe(CN)_6]^{4−}\) unit. The Fe\(^{3+}\) peaks of the PB crystals were only observed after 60 min. This evidence confirms that the initial reaction mainly produces PPy, and after sufficient diffusion of Py into the support pores, the crystallisation of PB is accelerated such that peaks assigned to both Fe\(^{3+}\) and Fe\(^{2+}\) are observed. The X-ray photoelectron spectroscopy (XPS) results demonstrate that the separation layer is composed of both PPy and PB, with PPy being the principal component. Hence, the concentration of Fe\(^{3+}\) may dominate the nanostructure of the separation layer because of its key bridge for connecting two reactions.

Non-damage blood separation

Owing to the direct contact of blood cells, the surface properties of the membrane are essential to prevent cell damage, otherwise interfering with the detection results after the intracellular components move out. To ensure the testing accuracy of the SepSen membrane in human blood, the risk of damage to blood cells must be evaluated. We confirmed the importance of the surface properties in providing a
biocompatible interface for direct contact with blood cells. In addition to water, serum contains many proteins and electrolytes that may also interact with the membrane surface, thereby affecting separation performance. Fig. 2e presents the surface hydrophilicity of the membrane to water and serum for comparison. The initial water contact angle was 77.4°, and the droplet rapidly permeated the pores within 3 s. If serum was added to the surface instead of water, the contact angle decreased to 58.5°. Moreover, the serum exhibited a slow permeation rate, which was evident by the presence of a liquid layer on the membrane surface at 30 s. This reveals the existence of an interaction at the interface between the membrane surface and other components instead of water in the serum. Although water accounts for approximately 90% of the serum composition, proteins play an essential role in wettability. Hydrogen bonds readily form between the lone pairs of electrons of the Py rings of PPy and the amino acids of proteins. Therefore, the proteins in the serum elicited transfer resistance to decelerate the permeation of serum into the membrane pores. This behaviour can provide a lubricant effect for blood cells flowing over the membrane surface, thereby reducing the possibility of cell damage. Moreover, the electric charge of the membrane surface was measured at pH 3–9. In such acidic to weakly alkaline conditions, the membrane surface was always negatively charged (Fig. 2f) according to the Gouy–Chapman equation as follows:

$$\sigma_d = -\kappa \xi \frac{F}{kT} \frac{e^{-\frac{F\Phi}{kT}}}{1 + e^{-\frac{F\Phi}{kT}}}$$

(4)

Where, k is the Debye-Hückel parameter, ξ (mV) is the membrane zeta potential, R is the gas constant (8.3145 J mol⁻¹ K⁻¹), F is the Faraday constant (96485 C mol⁻¹), Φ is the absolute temperature, and ε (6.933×10⁻¹⁰ F m⁻¹) is the permittivity. At pH 7.4, which is normal for human blood, the charge density reveals a high value of 1.52 mC m⁻². Owing to the negative surface charge of red blood cells (RBCs), the SepSen membrane surface, which has a strongly negative charge density, can prevent cell surface damage via electrostatic repulsion. These favourable properties are observed during in situ monitoring of RBC morphology during the separation process. After applying a vacuum driving force on the membrane, RBCs can continue to move freely on the membrane surface without apparent damage or morphological changes (Fig. 2g).

Among the various components to be separated from blood, FIB is the smallest with a molecular weight of 340,000 Da. To achieve total and precise rejection of white blood cells (WBCs), RBCs, platelets (PLTs), and FIB, the membrane pore size should be smaller than but approximately 340,000 Da. We found that by controlling the Fe³⁺ concentration in the precursor solution for membrane preparation, the pore size of the SepSen membrane could be effectively adjusted to directly control the molecular weight cut-off (MWCO) of the separation channel (Fig. 3d). At the Fe³⁺ concentration of 1 mM, the MWCO of the SepSen membrane can reach 315,000 Da, thereby satisfying the requirement for serum separation from whole blood. The separation behaviour is strongly confined by the pore size, which further affects the sensing process. If the size is much larger, the selectivity will be poor to hardly obtain pure serum. Conversely, a much smaller pore size will reject many proteins to retain only water, ions, and small molecules. This is because the Fe³⁺ ion serves as the initiator of the polymerisation reaction, whereas its reduced state is also one of the reactants for PB formation. We have already demonstrated that the rate of PB synthesis is lower than that of PPy generation, which is the control step for membrane preparation. In this case, a higher Fe³⁺ concentration can produce more PPy and PB to promote their intergrowth, thereby resulting in smaller pores. Consequently, all blood cells and FIBs were removed with no influence on other serum substances. Upon further increasing the concentration of Fe³⁺ ions during membrane formation, the MWCO suddenly decreased to less than 50,000 Da. As shown in Fig. S3, many PB nanocubes were generated on the membrane surface with poor uniformity that easily blocked the pores to enhance the resistance of serum separation, as well as increase the risk of cell damage.

**Versatile assays of various blood biomarkers**

PB is an excellent electrocatalyst for H₂O₂, which is the main product of oxidase reactions. Therefore, the membrane is capable of the versatile detection of different physiological substances in blood. To evaluate the biosensing performance, glucose and lactate oxidases were each immobilised in the biosensing channel through vacuum drawing from the lumen side to the outer surface of the SepSen membrane. These two oxidases promote the catalysis of the glucose and lactate substrates to produce H₂O₂, which is recognised by the electrocatalytic ability of PB. Therefore, these bio-recognitioners were confined in the nanopores, which was greatly beneficial for strengthening the catalysis and magnifying the detection signal. As shown in Fig. 3e, under electrochemical scanning from -0.2–0.4 V, the oxidation process was produced in the membrane. A typical oxidation peak was revealed at ca. 0.1 V, generated by the electron transfer from Fe(II) to Fe(III) in the PB unit cell to catalyse H₂O₂. In this process, the membrane can magnify the detection signal of the enzymatic reaction through its electrochemical redox. For the glucose test, the membrane exhibited a linear relationship for current versus concentration from 2.0–20.0 mM with a high sensitivity of 31.90 μA mM⁻¹ and a limit of detection (LOD) at 5 μM. Similarly, a broad linear range between 0 mM and 14 mM was achieved for lactate detection with a high sensitivity of 25.97 μA mM⁻¹ (Fig. 3e and f), as well as an LOD of 7.5 μM. We also tested the potential of the SepSen membrane as a versatile assay platform for analysing other physiological indices (Fig. 3g). By substituting glucose oxidase with glutaminate oxidase, the membrane can detect blood glutamate, which is relevant for evaluating neurological diseases, and reveals a linear detection range from 0–21 mM with a sensitivity of 24.16 μA mM⁻¹. Its LOD can reach 10 μM to present an evident response signal. The elevation of blood glutamate levels often indicates the possibility of Alzheimer’s disease.

In addition to detecting small molecules in blood, if we replaced PPy with glycyrrhizic acid to combine with PB as the nanocomposite (Fig. S4a), the membrane was capable of bonding with L-alanine and α-ketoglutarate, which can convert alanine aminotransferase (ALT) to glutamate recognised by glutamate oxidase in the membrane. To achieve this purpose, glutamate oxidase, L-alanine, and α-ketoglutarate were immobilised together in the membrane channel to produce the reactions. Therefore, this membrane device could be used to directly recognise blood ALT, which is one of the two major indices in whole blood for the diagnosis of various liver diseases.
The linear range for ALT detection is from 0–165 U.L−1 with a sensitivity of 54.6 nA U−1.L and a LOD of 0.2 U.L−1, which satisfies clinical requirements (normal healthy ALT level is below 50 U.L−1). The detection of glucose, lactate, glutamate, and ALT is based on enzymatic reactions in the SepSen membrane. The membrane can also be further modified to adopt the principles of immune and DNA biosensors. Using a Ni(en)3Ag2I4–graphene nanocomposite, the blood cells and FIB can also be rejected by the porous surface, which was mainly constructed by the stack of graphene sheets. In addition, Ni(en)3Ag2I4 functioned as an anchor to immobilise specific DNA strands and antibodies. Thus, the alpha-fetoprotein (AFP), which serves as an essential cancer biomarker to assess liver cancer[25], can be captured by the immune reaction to generate signals. The DNA-labelled antibodies can serve as the bridge for connecting AFP and Ni(en)3Ag2I4 to produce the signal (Fig. S5). The signal magnification relied on the electrochemical redox of Ag in Ni(en)3Ag2I4 at a work potential of 0.25 V. This membrane exhibited a linear detection ranging from 0–76 ng.L−1 with an LOD of 33 pg.L−1 through the reaction between AFP and monoclonal antibody. More importantly, in our previous work[26], the oriented Ni(en)3Ag2I4 crystals enabled the rapid recognition of the H5N1 virus because of the specific pairing of the DNA aptamer, thereby providing a potential for immediate early screening of virus infection in blood. The above-mentioned sensitive detection of five analytes is attributed to the confined effects of nanochannels, whereby regular crystals can serve as many microprobes to promote electrocatalysis on enzyme reactions, DNA, or antibodies[27].

Clinical performance in different medical scenarios

As expected, the heterogeneous nanostructure of the SepSen membrane can immediately separate whole blood on its top separation layer and output the detection signal of the target concentration by its biosensing channels while in contact with the whole blood. Using the membrane device in Fig. 1a, we simultaneously recorded online, two critical performances of serum permeance and current signal using human blood to simulate the continuous surgery process to evaluate the possibility of dynamic detection. During the blood analysis, both the detection signal and the flow rate of the separated serum were rapidly stabilised (Fig. 4a), such that the test results could be generated in ca. 1 min, which was much faster than the traditional blood assay technology. Importantly, the response current can be continuously output to present the fluctuation of the analyte concentration during blood drawing; thus, confirming its online and dynamic monitoring ability for key analytes during a period of operation. This rapid stabilisation indicates that the isolated serum flows quickly and continuously into the biosensor channel and immediately produces the response current signal.

Figure 4. Clinical performance of the separation–sensing (SepSen) membrane device to patients from a variety of medical scenarios (a) Online recordings of the biosensing current signal (red line) and the dynamic separation rate (blue line) during simultaneous separation and glucose analysis of patient blood. (b) Digital photographic (left) and microscopy (right) images of whole blood (top) and serum (bottom) separated using the device. (c) Separation performance of blood cells (white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs)), fibrinogen (FIB), the vital ions Na+ and K+, as well as the analytes, i.e., glucose and lactate. (d) Glucose and lactate detection by the as-prepared membrane device ('membrane') in blood samples from five patients in different medical scenarios, and comparisons with hospital assay results ('instrument').
As expected, by typical observation of the separation process, the collected serum is transparent and light yellow in colour, whereas whole blood is red and opaque (Fig. 4b). A serum sample was further analysed to confirm that it did not contain WBCs, RBCs, PLTs, or FIB (Fig. 4c, table). Notably, compared with the original whole blood (Fig. 4c, analysed by means of the commercial instrument), the serum contained near-identical concentrations of two major electrolytes (Na" and K") and two detection targets (glucose and lactate). The amount of intracellular potassium can reach approximately 98% of the total amount in the body with concentrations between 140 mM and 150 mM; however, the extracellular potassium level is only approximately 3.5–5 mM. If the blood cells are damaged and rupture on the membrane, ion concentration increases dramatically. This demonstrates the excellent biocompatibility of the SepSen membrane, which is attributed to its advanced surface wettability and electrostatic interactions. Because glucose and lactate are the target analytes, the consistency of their concentrations before and after separation is the basis for the accuracy of the analysis. Furthermore, the levels of glucose and lactate in the blood samples collected from five patients (no diabetes and hyperlactatemia) with different medical conditions/procedures (liver transplantation as a cancer treatment, emergency portal vein thrombosis, gallbladder polyps, endometrial cancer, and right femoral neck fracture) during their surgeries were determined using this device (Fig. S7). All the results gathered using the SepSen membrane device were consistent with those of the commercial biochemical instrument, which inevitably required centrifugal separation of whole blood in the hospital.

During liver transplantation, significant increases in both glucose and lactate typically occur as the organ is removed. Our device accurately detected this change in the patient as the simultaneous elevation of both concentrations above normal levels in a healthy patient (Fig. 4d). A patient with portal vein thrombosis showed ultrahigh blood concentrations of glucose and lactate, and the device produced results within 1 min; this can take as long as 20 min with conventional analysis methods based on centrifugal blood separation. As for the other three conditions (gallbladder polyps, endometrial cancer, and right femoral neck fracture), minor deviations from levels of a healthy subject often occurred for glucose or lactate during surgery, which were also timely and accurately captured by the SepSen membrane device with little interference.

**Conclusion**

We present a new platform for dynamic blood diagnosis using a separation-biosensing membrane for real-time and continuous testing of various essential serum components during blood drawing through its heterogeneous nanostructure, which possesses a surface nanoporous separation layer and a regularly-structured biosensing layer underneath. The analytical compatibility of the device with the clinical instruments used for patients suffering from different medical conditions and the onsite and dynamic detection mode provides great potential for online monitoring of the concentration fluctuation of fatal indicators over time during surgery and emergency treatment to reduce risk, which is an extremely powerful but unrealised technique in clinical medicine. Additionally, the excellent compatibility of the device with different materials, synthetic methods, and principles of analysis might pave the way for new and interdisciplinary research in membrane science, analytical chemistry, biology, and clinical science.

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**Keywords:** hollow fibre membrane • heterogeneous nanostructure • real-time analysis • blood separation • electrochemical biosensing

A separation-sensing membrane is constructed into a heterogeneous nano-architecture wherein a nanoporous surface layer continuously extracts serum and the biosensing nanochannels underneath dynamically detect the biotarget fluctuation, thereby achieving online and dynamic tests of various vital clinical indices during blood drawing in patients from a variety of medical scenarios, including liver transplantation and endometrial cancer.