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Study of inflammatory factors' effect on the endothelial barrier using piezoelectric biosensor

Junliang Han[a], Feifei Tong[b,*], Ping Chen[b], Xiancai Zeng[b], Zhengyong Duan[a]

[a] School of Mechanical and Automotive Engineering, Nanyang Institute of Technology, Nanyang 473000, China
[b] School of Biological and Chemical Engineering, Nanyang Institute of Technology, Nanyang 473000, China

A B S T R A C T

This paper used piezoelectric sensor to study the dysfunction of endothelial cell monolayer barrier caused by inflammatory factors. The biocompatible conductive polymer membrane of pPy[pGlu]-pLys was prepared on the surface of the ITO work electrode to improve the interface between the endothelial cell and the electrode. Both the impedance analysis data and the stable plateau stage of sensor's frequency shift indicated that endothelial cells formed a good monolayer barrier on this polymer surface. The response frequency shifts of lipopolysaccharide (LPS)- and histamine-induced endothelial barrier dysfunction were different, which distinguished their different stimulation mechanism. It provided a valuable analysis method for detecting the endothelial barrier function affected by inflammatory factor, and could further promote the application of piezoelectric sensor in cell biology and toxicology research.

1. Introduction

The endothelial cells are lined to blood vessels. The integrity of vascular endothelial barrier plays an important role in maintaining blood stable circulation and the function of tissues and organs (Edens and Parkos, 2003). Under physiological conditions, the endothelial cells not only grow and proliferate, but also secrete a variety of extracellular matrix proteins, such as cadherin, occludin, elastin and fibrin, etc. These mutually inter-linked proteins constitute basal membrane, which only allows small molecules go through freely and restricts the passing of macromolecular substances. It serves as a barrier between blood and tissues. The dysfunction of endothelial cell barrier usually reflects pathological inflammatory reaction firstly, and it is also closely related to the severity of disease (Bone et al., 1997; Peters et al., 2003).

Analysis and evaluation method of the endothelial cells' barrier function is performed by the permeability measurements. It mostly used membrane filter to detect transmittance of the marker (Fluorescein isothiocyanate-dextran, FITC-dextran) through endothelial monolayer (Van Nieuw Amerongen and van Hinsbergh, 2002; Ammori et al., 1999). These detections are complicated, and can only analyze endothelial permeability at certain time point. While the physiological and pathological changes of endothelial cells are dynamic processes. Therefore, we proposed to track these processes in real-time using sensor. The piezoelectric sensor has many advantages in monitoring cell dynamic processes (Tong and Lian, 2014; Tong et al., 2015, 2016). It is label-free, and carries out quantitative and dynamic monitoring. So it is suitable for studying the barrier functions of endothelial cells.

To study the barrier function of endothelial cells by sensor method, it is necessary to lay endothelial monolayer in good growth state on the surface of the sensor's sensitive electrode. In order to simulate endothelial cells' physiological conditions in vivo, and make more accurate evaluation of the effect of inflammatory factor, biocompatible conductive material can be adopted to modify the work electrode surface. It can not only be used as the biomimetic membrane of endothelial cell basement, but also reflect the signals of cell, which is conducive to studying the effect of stimulants on the barrier function of endothelial cells. The use of pLys as the substrate film on gold piezoelectrodes for designing biosensors had been explored (Stobiecka and Hepel, 2011). The biosensors utilizing cells and organelles had also been developed to evaluate their responses to various factors. A piezoelectric sensor with immobilized mitochondria was designed for measuring ion flaxes via potassium channels in hypotonic and hypertonic solutions, as well as under the influence of valinomycin (Magdalena et al., 2017).

The conductive polypyrrole doped with long-chain biomolecule had good biological compatibility and could enhance nerve cell's adhesion to basement. It was used to lead nerve cells to grow into the micropattern as designed in advance (Kim et al., 2010). In this study we adopted the biocompatible and conductive polymer membrane to modify the electrode of piezoelectric sensor. The preparing procedures of polymer membrane and endothelial cell monolayer were

* Corresponding author.
E-mail address: tfeifei99@hotmail.com (F. Tong).

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demonstrated by electrochemical impedance spectroscopy (EIS). The charge transfer resistance and capacitance of each modified layer were consistent with the electrical properties on the same layer. Series piezoelectric sensor with modified electrodes was used to measure the influences of lipopolysaccharide (LPS), histamine and other exogenous or endogenous inflammatory substances on the barrier function of endothelial cells. The results had shown that piezoelectric sensor with electrode modified by biocompatible and conductive polymer membrane was a good method to evaluate the inflammatory damage to the barrier function of endothelial cells.

2. Experimental section

2.1. Materials and reagents

Pyrrole; poly-glutamate (pGlu, MW = 17,000 by viscosity, about 100 monomers); poly-L-lysine (pLys, MW = 70,000–150,000); 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC); *N*-hydroxysuccinimide (NHS); 2-[*N*-morpholino] ethane sulfonic acid (MES) and bovine serum albumin (BSA) (Sigma-Aldrich). LPS and histamine were dissolved in PBS buffer solution and sterilized by filtration. All other reagents used in the experiment were AR grade. The water was ultra pure.

2.2. Apparatus

CHI6000B electrochemical workstation (Chenhua, shanghai); HP4192A impedance analyzer (Hewlett-Packard, USA); Biological phase contrast microscope (CKX41, Olympus, Japan). Indium tin oxide (ITO) semiconductor slides with the conductive layer thickness and surface resistance of 78.5 ± 1.5 nm and 20.6 ± 2.5 Ω/cm^{-2}, respectively, were obtained from Shenzhennanbo, China. The piezoelectric biosensor was self-developed based on the principle of series piezoelectric response in our laboratory. It consisted of culturing-detection system, oscillating circuit, microprocessor and computer operating system as shown in Fig. 1.

2.3. Preparation of biologically compatible conductive polymer membrane

The biologically compatible conductive polymer membrane on electrode surface was prepared by two steps of electrodepositing and chemical cross-linking. Electrodepositing was conducted by cyclic voltammetry (CV). 400 μL pyrrole monomer (200 mM) and 200 μL pGlu solution (2 mM) were added into the sensor culturing-detection cell which basement was indium tin oxide (ITO) electrode. The supporting electrolyte was sodium para benzene sulfonate solution (pH = 7). Connect the sensor's electrode leads to CHI6000B electrochemical workstation and select three-electrode system. The ITO electrode at the bottom of detection cell worked as working electrode, the platinum wire as counter electrode, and the saturated calomel electrode (SCE) as reference electrode. The polypyrrole (pPy) film doped with pGlu was electrodeposited on the surface of the ITO electrode by scanning the potential between 0.0 and 1.0 V for 10 cycles at a rate of 100 mV/s. The temperature should be controlled at 5 ℃ in reaction process.

Poly-L-lysine chain was chemical cross-linked with pGlu chain which exposed outside of pPy film by synthesizing amide bond in EDC/NHS catalytic reaction. Carefully suck out the previous reaction's residues and then rinse the culturing-detection cells with sterile water for three times. 100 μL each of 0.5 mg/mL EDC, 0.5 mg/mL NHS, 0.1 M MES and 0.5 M NaCl solution was mixed uniformly and added into the cells. Incubate it for 30 min at room temperature to synthesize PPy [pGlu]-NHS which could activate the surfaces of polymers film. Then carefully suck out the residues, rinse the culturing-detection cells again. Add 200 μL pLys solution (100 μg/mL) and incubate it for 2 h at RT to synthesize PPy[pGlu]-pLys film which was the biologically compatible conductive polymer membrane on the surface of the sensor electrode. Sterilize it under ultraviolet light for 10 min, and then rinse the culturing-detection cell for many times. Before seeding endothelial cells, Incubate it with culture medium containing BSA for 0.5 h.

2.4. Measurement of the electrochemical impedance

The modification of the electrode by pPy[pGlu] or pPy[pGlu] and the cell growth on the surface of the polymer membrane would cause changes in the interfacial impedance. It was measured by electrochemical impedance with three electrode system. The sensor's detection panel electrode leads were connected to the HP4192A impedance analyzer. ITO electrode modified by polymer membrane worked as working electrode, platinum wire as counter electrode, SCE as reference electrode, and K3Fe(CN)6/K4Fe(CN)6 (5 mM , 1:1) as the redox probe. Scanning frequency range was in 5–13 Hz. And sinusoidal voltage amplitude was at 10 mV. The impedance spectroscopy of electrode with different modification was measured in culture solution (pH7.8). Zsimp Win was performed for fitting the data.

2.5. Preparation of endothelial cells monolayer

Human umbilical vein endothelial (HUVEC) cells were collected by trypsinization (0.25% trypsin/0.02% EDTA) and suspended in low-glucose DMEM medium containing 10% FBS. The suspended cells were seeded in the sensor's culturing-detection cell with a density of 1×10^5cell/mL and the detection electrode were connected with instruments. Then the detection plate was put in incubator at 37 ℃. The growth curve of HUVEC cells was detected and recorded by computer. After 72 h of culturing, HUVEC cells attached to the whole bottom of the culturing cells and covered the surface of electrode forming

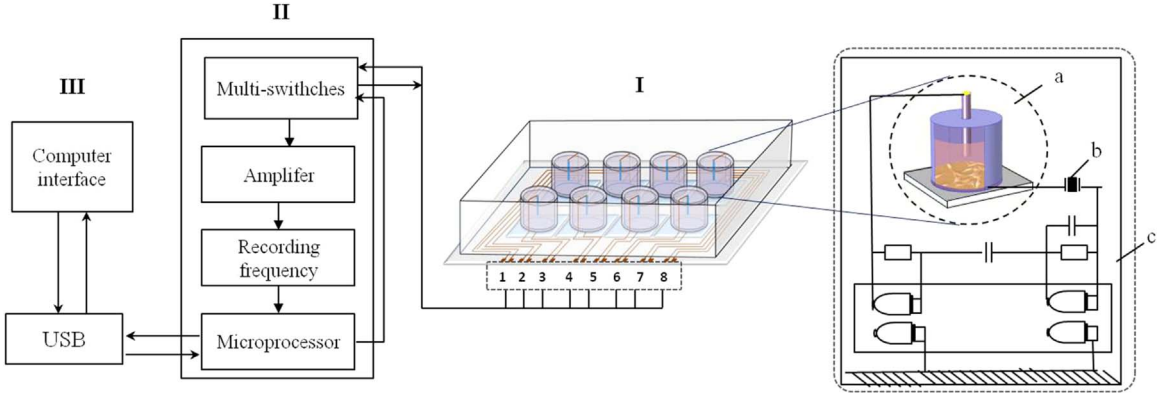

Fig. 1. Schematic diagram of the piezoelectric biosensor. (I) Cell culture-detection system (a is the culture cell; b is the 9 MHz AT-cut quartz crystal; c is the oscillating circuit); (II) Data processing system; (III) PC interface.