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Cytocompatibility of poly(acrylonitrile-co-*N*-vinyl-2-pyrrolidone) membranes with human endothelial cells and macrophages

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Abstract

Polyacrylonitrile modified with *N*-vinyl-2-pyrrolidone (NVP) shows good hemocompatibility. This work, which aims to evaluate the cytocompatibility of membranes fabricated from poly(acrylonitrile-co-*N*-vinyl-2-pyrrolidone) (PANCNVP), studied the adhesion of macrophages and endothelial cell (EC) cultures. It was found that PANCNVP membranes with higher NVP content decreased the adhesion of both macrophages and ECs. Compared with polyacrylonitrile and tissue culture polystyrene control, however, these PANCNVP membranes promoted the proliferation of ECs. Furthermore, the viability of ECs cultured on the PANCNVP membrane surfaces was also relatively competitive. Both static and dynamic water contact angle measurements were conducted to explain the nature of cell adhesion to the PANCNVP membranes. On the basis of these results and the phenomena of water swelling and water states reported previously, it was presumed that the coexistence of large amounts of bound water and free water induced by NVP moieties are responsible for the lower adhesion and better function of cells adhering to the PANCNVP membranes.

Keywords: Membranes surface; Cytocompatibility; Endothelial cell; Macrophage; Copolymer

1. Introduction

Poly(*N*-vinyl-2-pyrrolidone) (PVP), a known water-soluble and biocompatible polymer, has been widely used as a blood plasma substitute, additive and surface modifier [1–12]. In particular, PVP ensures excellent hemocompatibility for many kinds of dialysis membranes, e.g. polyacrylonitrile and polysulfone membranes. PVP is usually blended with other polymers for membrane preparation, but its subsequent elution from the membrane is disadvantageous during hydraulic permeation [8]. Therefore, we synthesized copolymers of acrylonitrile and *N*-vinyl-2-pyrrolidone (NVP) by a simple process, and our previous work has confirmed the hemocompatibility of the corresponding copolymer membranes [13,14]. The introduction of NVP into polyacrylonitrile (PAN) can suppress blood platelet adhesion and increase plasma recalcification time as well as reduce bovine serum albumin adsorption on the PAN-based membranes. In addition, these copolymers have superior membrane-forming properties. Therefore, this type of membrane appears suitable for hemodialysis.

Generally speaking, microporous polymer membranes, except those being considered for tissue engineering, should inhibit protein adsorption as well as cell adhesion [15]. It is believed that the behavior of cells on a specific biomaterial surface may vary greatly with the type of cell investigated [15,16]. Macrophages are a kind of immune cell and perform various functions in living bodies, such as migration, phagocytosis, secretion, antigen presentation and survival through precisely modulated adhesion [17,18]. Preventing the activation of immune cells is a requirement for biomedical membranes. In contrast, endothelial cells (ECs) constitute the natural antithrombotic surface contacting the

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blood in native vessels. Endothelialization is an approach to produce an antithrombotic surface for blood-contacting devices, such as vascular grafts. Many efforts have been made to generate EC-seeded surfaces, including surface plasma treatment [19], patterned or phase-separated surface construction [20–24], protein/peptide coating or immobilization [25–35], chemical modification [36,37] and other methods [38–40]. However, results show that blood platelet adhesion to the endothelialized surface is affected not only by the seeding of ECs but also by the underlying substrate. The nature of the substrate affects the coagulant functions of ECs, such as the production of platelet-activating factor and tissue plasminogen activator [41]. Therefore, studying the interactions of materials with ECs that have special significance for hemocompatibility may help in understanding the hemocompatibility of such materials. In this work, macrophage adhesion and EC culture were performed to evaluate the cytocompatibility of poly(acrylonitrile-co-*N*-vinyl-2-pyrrolidone) (PANCNVP) membranes.

2. Materials and methods

2.1. Membrane preparation

PANCNVP and PAN were synthesized in our laboratory. Details of the synthesis and characterization of the polymers were described previously [13,14]. The NVP content of the copolymers, calculated from ^{1}H NMR spectra, was 7, 15, 22 or 31 wt.% and those copolymers were denoted as PANCNVP7, PANCNVP15, PANCNVP22 and PANCNVP31, respectively. Membranes were prepared by casting the polymer solutions in DMF (8 wt.%) onto clean glass plates which were then dried for 24 h at 100 °C under vacuum to remove the residual solvent. After immersion in pure water for another 24 h, the resultant membranes were finally dried at 60 °C under vacuum. All membranes for contact angle measurement, macrophage adhesion and EC culture were treated according to the same procedure. The thickness of these membranes was approximately 18 ± 2 μm.

2.2. Water contact angle measurement

The water contact angles of the membranes were measured by the sessile drop method with a contact angle goniometer (OCA20, Dataphysics, Germany) at room temperature. In a typical sessile drop method, a water drop (~5 μl) was placed on the dry membrane surface and the static water contact angle was determined after 10 s. The dynamic contact angle – in our case, the dependence of contact angle on time – was recorded every 30 s for 30 min.

2.3. Macrophage adhesion

The murine macrophage suspension was prepared using the method reported previously [17]. This suspension was isolated from freshly killed mice. After killing the animals with chloroform, the skin was sprayed with alcohol and the abdomen was opened. A 10 ml sample of Roswell Park Memorial Institute (RPMI) 1640 containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin was injected into the peritoneal cavity, and then the abdomen was gently massaged manually for 5 min. The peritoneum was carefully punctured, and then the washings were removed with a sterile pipet and placed in a sterile container which was centrifuged at 1000 rpm. for 10 min to collect the macrophages. The macrophages were grown in RPMI 1640 to obtain a macrophage suspension in which the cell concentration was 1×10^6 cells/ml.

The membrane (10×10 mm^2) was cleaned sequentially in an ultrasonic bath of ethanol solution for 10 min and rinsed in PBS. The sample was then immersed in physiological saline (pH 7.4) to recondition for 2 h. The cell suspension was inoculated on the surface of the membrane to assess the cell adhesion. The incubation period was 48 h for the cell adhesion test in a humidified atmosphere of 5% CO_2 in air at 37 °C. The supernatant was then removed, and the membrane was washed cautiously five times using PBS (pH 7.2), and the adherent cells were fixed by the addition of methanol for 5 min. The adherent cell density on the membrane was quantified on the basis of measurements obtained visually from at least five randomly selected fields (0.24×0.36 mm^2) using an Olympus TE300 phase-contrast optical microscope. The mean values and standard deviation of triplicate samples for each membrane were reported.

2.4. Endothelial cell culture

Human umbilical vein ECs were isolated from the human umbilical cord veins with 1.0 mg/ml collagenase (type I, Sigma)/phosphate buffer solution (PBS, pH 7.4) for 20 min at room temperature. The isolated ECs were routinely seeded at a density of 100,000 cells/cm^2 on the membranes laid in the cell culture well. As a control, the ECs were also directly seeded on tissue culture polystyrene (TCPS, Nunc, Denmark). The ECs were incubated in a culture medium consisting of 20% (v/v) FBS and 80% RPMI 1640 supplemented with 100 U/ml of penicillin and 100 μg/ml of streptomycin in humidified air containing 5% CO_2 at 37 °C. The morphology of living cells after 96 h of incubation was observed under an optical microscope (BX51T-3200, Olympus).

After 12 h and 4 days of incubation, the medium was discarded. The samples were washed three times with PBS, which removed the poorly adhering and suspended cells. Then 100 μl 0.25% trypsin solution was added to each well. After a 15 min digestion, 100 μl of media was added and the cells were resuspended. The cells were counted with a hemocytometer and a flow cytometer. The adhesion density of cells on the TCPS surface was used as a standard. We define the cell adhesion ratio at $t = 12$ h or 4 days as

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