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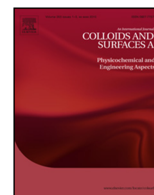

Superhydrophobic/hydrophobic nanofibrous network with tunable cell adhesion: Fabrication, characterization and cellular activities

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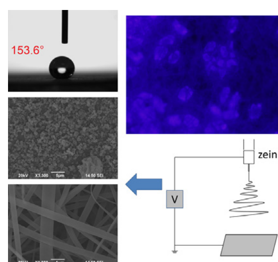
HIGHLIGHTS

- Zein superhydrophobic/hydrophobic nanofibrous network was formed by electropinning.
- The highest WCA of the zein electrospun nanofibrous network (ZENN) could reach 153.6°.
- Both the zein concentration and applied voltage had effects on the surface hydrophobicity.
- ZENN could mimic the extracellular matrix to support the cell growing.

GRAPHICAL ABSTRACT



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ABSTRACT

Superhydrophobic/hydrophobic nanofibrous networks have attracted much attention because of their potential applications in tissue engineering. Cell growth in the scaffold of tissue engineering can be controlled by the hydrophobicity of the scaffold. The superhydrophobic/hydrophobic surfaces are usually made from synthesized polymers, which generally are not biocompatible and biodegradable and, thus, not suitable for biomedical applications. Zein is an amphiphilic protein from corn, and it is potential for hydrophobic surface formation. This work aims to make zein superhydrophobic/hydrophobic nanofibrous network using electrospinning. The formed zein networks show high hydrophobicity with the water contact angles ranging from 130.5 ± 1.0° to 153.6 ± 2.1°. The cell attachment and growth on the zein networks are studied. It is observed that the amount of the cells attached and grown in the zein nanofibrous networks are higher than the ones on the conventional zein casting films. The results indicate that the electrospun zein nanofibrous network has great potential as scaffold in tissue engineering to support cell growth and tissue regeneration.

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1. Introduction

Hydrophobic surface is a surface exhibiting water contact angles (WCA) higher than 90°. It has wide applications in coating, textile, packaging, electronic devices, and biomedical engineering [1]. Superhydrophobic surface is a special kind of hydrophobic surfaces with a WCA higher than 150°. Superhydrophobic surface has attracted much attention over the past decades for its applications

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in many areas including microfluidics [2] and self-cleaning surfaces [3]. Hydrophobic surfaces play an important role in biomedical engineering, especially tissue engineering. Tissue engineering is aiming to create biochemical and physico-chemical substitutes to improve or replace the biological functions of portions or the whole tissue of human body. In tissue engineering, a scaffold is required for the incorporation of living cells and to support the cells to adhere, grow, and differentiate. The hydrophobicity and roughness of the surface as well as the microenvironment in the scaffold affect the cell attachment and growth [4]. For example, Valamehr et al. [5] had confirmed that hydrophobic surfaces promoted the proliferation and differentiation of the stem cells.

Generally, there are two ways to improve the hydrophobicity of a surface: increasing the surface roughness and reducing the surface energy [6]. Surface roughness at nano- and micro-meter scales can be improved through a variety of methods, such as lithography, chemical etching, templating, sol–gel synthesis, controlled crystallization, and phase separation [7]. Hydrophobic surfaces can be formed on various substrates, such as synthetic polymers, Si wafers, glass slides, and metals [8]. However, these substrates are usually too rigid for biomedical applications [9]. A loss of hydrophobicity may also occur when the substrates deform [10]. Moreover, for biomedical applications, high biocompatibility and low toxicity are required. Therefore, Foreign synthetic materials are extremely limited for such uses. Natural plant-based biomaterials are biocompatible, low/non-toxic, inexpensive, sustainable, and biodegradable [11]. However, most of the natural biomaterials are hydrophilic and water absorbing. They may be rapidly solubilized in aqueous environment, which is not good for tissue engineering applications [12].

Zein is a major protein extracted from corn endosperm. It can be dissolved in 40–95% ethanol–water mixture [13]. Zein is capable of self-assembly into various structures, such as microspheres, bicontinuous sponges, films, and fibers [14]. Zein has more than 60% of hydrophobic amino acids, which makes it amphiphilic and highly potential for hydrophobic surface formation. Zein, as a natural biopolymer, has advantages over manufactured synthetic polymers, such as good biodegradability, low toxicity, and high biocompatibility, and those are especially important for applications in biomedical engineering areas. In addition, different from other natural biopolymers, zein is not water-absorbing.

In this paper, we report the fabrication of zein superhydrophobic/hydrophobic nanofibrous network using electrospinning. We investigate the effects of zein concentration and the electrospinning voltage on the surface hydrophobicity of the zein electrospun nanofibrous network (ZENN). WCA and SEM are used for characterizations. The results indicate that the ZENN could mimic the extracellular matrix (ECM) to support the cell growing and are highly potential for tissue engineering applications.

2. Materials and methods

2.1. Materials

Zein was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Ethanol (96% v/v) was obtained from Guangdong Guanghua Sci-Tech Co. Ltd. (Guangzhou, China).

2.2. Electrospinning

Zein solutions of various concentrations (5, 10, 15, 20, 25, and 30 wt%) were prepared by dissolving zein in 80% (v/v) aqueous ethanol solution followed by 10-min sonication. A nanofiber electrospinning unit (Kato Tech Co., Ltd., Tokyo, Japan) with a variable high voltage power supply of 0–40 kV was used. The positive electrode was attached to a metal needle with an internal diameter of 0.9 mm connected to a 10-ml syringe filled with zein solution. The syringe was placed horizontally on a controlled syringe pump and the flow rate was kept at 0.5 ml/h. And the needle was horizontally directed towards the collector, which is rotating at 1200 rpm. Under the applied voltage of 10–24 kV and with the tip-to-collector distance of 15 cm, a positively charged jet of zein solution was formed, traveled through the air gap, and deposited on the collector covered with an aluminum foil. After electrospinning for 2 h, the samples (0.7 mm thick) were removed from the collector.

2.3. Water contact angle (WCA)

WCA was used to measure the surface hydrophobicity. A hydrophobic surface has a WCA larger than 90° and a superhydrophobic surface has a WCA larger than 150°. WCA was measured using a standard goniometer (Kruss GmbH DSA 100, Hamburg, Germany). The water droplets were introduced by a micro-syringe, and images were captured using a camera system. The WCAs were calculated according to the Young–Laplace equation by the software.

2.4. Scanning electron microscopy (SEM)

The surface morphology of the ZENN was examined using SEM. The ZENN were gold coated using an Edwards S150B sputter coater to improve the electrical conductivity. SEM images were obtained using a JEOL JSM-6490 SEM (Tokyo, Japan).

2.5. Oxygen plasma treatment

Oxygen plasma was used to introduce the oxidation of the hydroxyl groups on ZENN surfaces to increase the surface wettability. A PDC-32G plasma cleaner (Harrick Plasma, USA) with low-pressure mercury vapor lamp was used to treat the ZENN. The ZENN was placed in a vacuum chamber, the pressure in which is 110–115 m Torr, and exposed to oxygen plasma for 5–10 min.

2.6. Cell culture

Human liver hepatocellular carcinoma cells (HepG2) and rat osteoblastic UMR106 cells were incubated in Dulbecco's Modified Eagle Medium (DMEM), which contains 100 mg/ml penicillin and 100 mg/ml streptomycin. The cells were supplemented with 10% fetal bovine serum (FBS), and maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO_2. Then, the cells were trypsinized using a 0.25% trypsin solution in PBS buffer for 5 min and resuspended in the complete culture medium.

2.7. Cell adhesion assay

The cell attachment assays of HepG2 and UMR106 were conducted on three samples, the ZENNs, the oxygen plasma treated ZENNs (OZENNs), and the zein casting films (ZCFs, 0.7 mm thick) were collected on a rotated collector covered with an aluminum foil, respectively. The ZENN was made from the zein solution (30 wt%). It was cut into pieces in the size of $10 \times 10\,mm^2$. Each ZENN sample was then attached on a microscope cover glass and hold in a 6-cell plate. The ZCF sample was prepared by cast drying of the 30 wt% zein solution. All the samples were sterilized using UV light for 30 min before the cell experiments. SEM was used for the cell adhesion study. For sample preparation of SEM, cells were seeded on the 6-well plate at a density of 2×10^5 cells/ml and allowed for cell attachment for 24 h. After incubation, the samples were washed three times by PBS and fixed by the 4% paraformaldehyde solution. The samples were freeze–dried

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