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Construction of a fucoidan/laminin functional multilayer to direction vascular cell fate and promotion hemocompatibility

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ABSTRACT

Surface biofunctional modification of cardiovascular stents is a versatile approach to reduce the adverse effects after implantation. In this work, a novel multifunctional coating was fabricated by coimmobilization of the sulfated polysaccharide of brown algae fucoidan and laminin to biomimic the vascular intimal conditions in order to support rapid endothelialization, prevent restenosis and improve hemocompatibility. The surface properties of the coating such as hydrophilicity, bonding density of biomolecules and stability were evaluated and optimized. According to the biocompatibility tests, the fucoidan/laminin multilayer coated surface displayed less platelet adhesion with favorable anticoagulant property. In addition, the fucoidan/laminin complex showed function to selectively regulate vascular cells growth behavior. The proliferation of endothelial cells (ECs) on the fucoidan/laminin biofunctional coating was significantly promoted. For the smooth muscle cells (SMCs), inhibitory effects on cell adhesion and proliferation were observed. In conclusion, the fucoidan/laminin biofunctional coating was successfully fabricated with desirable anticoagulant and endothelialization properties which show a promising application in the vascular devices such as vascular stents or grafts surface modification.

1. Introduction

The widespread application of cardiovascular implants such as blood vessel stents, interventional catheters and artificial heart valves saves patient's lives and improves their life qualities to a high degree [1]. But complications after implantation due to thrombosis [2], late endothelialization [3] and neointimal proliferation [4] still occur frequently and cause a failure of the therapy. In order to overcome these problems, many measures have been taken to improve implants biocompatibility so as to reduce the adverse interactions between the material and the blood vessel. One of the promising technologies is surface modification of the implant, with the aim to create a microenvironment similar to biology and thus to regulate the proliferation behavior of vascular cells [5]. In the normal blood vessel, the intima, a monolayer of endothelial cells (ECs) is the key protection against thrombosis, and it maintains the vascular homeostasis. The underlying nanofibrillar basement membrane supports endothelium growth and controls the proliferation of vascular smooth muscle cells (VSMC). The support of rapid regeneration of the endothelium and maintenance of the intimal stability are the latest requirements for the functionalization of cardiovascular biomaterials [6,7].

A variety of modification approaches such as plasma treatment [8], surface nanostructuring [9], and chemical immobilization of extracellular matrix components [10] have been applied to modify the surface properties of cardiovascular devices, including ceramic coatings, metallic alloys and polymer coatings. These methods have been developed to construct specific non-thrombogenic microenvironments, which support re-endothelialization, and prevent SMC proliferation. However, these techniques focus on only one aspect of improving biomaterial/tissue interaction. An ideal implant surface should own multiple functions like the vascular intima to effectively protect vascular homeostasis from adverse effects after implantation.

In the process of constructing a surface with multiple functional properties, growth factors (VEGF/HIF/PDGF/bFGF) [11], adherent proteins or peptides (fibronectin/laminin/RGD) [12,13] and polysaccharides (hyaluronic acid/heparin) [14,15] are the most promising candidates for biomimetic manufacture. These biomolecules are known to interact with proteins, cells and growth factors which participate in thrombosis, inflammation and tissue remodeling. Fucoidan (F) is a sulfated polysaccharide, with a molecular weight range of 2000–20,000 Da originally from brown algae. In the past decade it has been extensively studied due to its numerous interesting biological activities, such as anticoagulant [16] and antithrombotic activity [17], angiogenesis [18], anti-inflammatory properties [19] etc.

Fucoidan has significant anticoagulant efficiency and is similar to the sulfate structure of heparin in the basal membrane. Heparin is regarded

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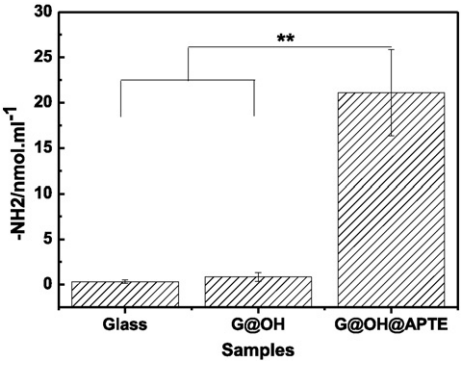

Fig. 1. Acid Orange II characterization optical density of Glass, G@OH and G@OH@APTE.

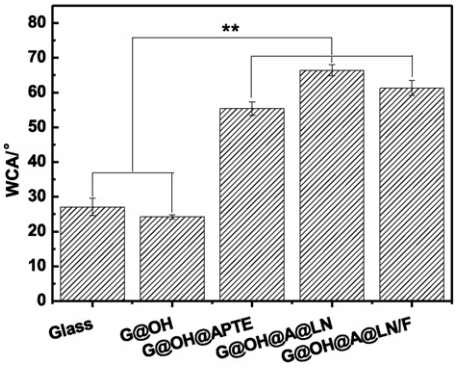

Fig. 3. Water contact angle measured on the samples of on glass, G@APTE and G@A@Ln/F were analyzed (mean ± SD, N = 3).

as a kind of multi-functional molecules with properties of anticoagulation, promotion endothelialization and inhibition of SMC proliferation [20,21]. But there is no systematic knowledge about the influence of fucoidan on blood vessels cells.

In the present work, we develop a multifunctional molecules film composed by fucoidan (F) and laminin (LN). As an important constituent of the basal membrane, Laminin plays a crucial role in various cell surface interactions, helping to mediate both cell-to-cell and cell-to-ECM adhesion and to promote cell differentiation and proliferation [22]. Here, an F–LN complex was covalently conjugated on the material surface. The molecule interaction between fucoidan and laminin maintains a film structure similar to the basement membrane with a 3D orientation recognized by the cells. We simultaneously expect that the F–LN modified surface possesses similar multifunctional properties as heparin for selective promotion of ECs proliferation, inhibition of SMC proliferation and blood platelet activation.

2. Experimental

2.1. Materials

Glass slides (10 * 10 mm) were cut using a glass knife. The glasses were ultrasonically cleaned twice successively with ethanol and deionized water (dH_2O) for 5 min each and then were dried in an oven at 60 °C for 2 h before use. 0.0067 M phosphate buffer saline (PBS, pH 7.3) was purchased from Hyclone Co. Ltd. H_2SO_4 and H_2O_2 were used as stop solution for immunochemistry assay. 3-Aminopropyltriethoxysilane (APTE), Toluidine Blue O (TBO) and Acid Orange 7 (AO) were purchased from Sigma-Aldrich.

Fucoidan (F, Product of USA) from *Fucus vesiculosus* was purchased from Sigma-Aldrich. Mouse laminin (Ln) from the basement membrane, cell counting kit (CCK-8) for cell culture and proliferation test were purchased from Sigma-Aldrich. All the other reagents used in the experiments were of the highest analytical purity (>99.9%).

2.2. Coimmobilization of Ln/F on glass substrate

The cleaned Glass plates were activated in H_2SO_4/H_2O_2 (H_2SO_4:H_2O_2 = 7:3) solution at 80 °C for 1 h, and then thoroughly rinsed with dH_2O and blown dry. This activation process produced a very hydrophilic substrate, which was then silanized by immersing into a 3% v/v solution of APTE in 99.8% anhydrous ethanol for 12 h to generate an amino surface. The APTE silanized glass surface was referred to as G@APTE and was sonicated in ethanol in order to obtain the silane monolayer and to remove the physisorbed molecules. After that, the samples were kept in a vacuum oven a 120 °C oven for 2 h to enhance the binding of APTES with the substrate [23]. 200 μg/mL fucoidan and 100 μg/mL laminin were pre-diluted in PBS at 37 °C for 1 h and then activated with EDC/NHS/MES at a volume ratio of 1:9. G@APTE samples were immersed into this activated Ln/F solution 37 °C for 2 h and rinsed with PBS to remove the non-conjugated fucoidan and laminin molecules [12]. Thereafter the sample was denoted G@A@LN/F.

2.3. Amine group quantification with Acid Orange II

In order to determine surface amine concentration after the binding of APTES with the substrate, samples with 10 * 10 mm were immersed into a 500 μmol/L Acid Orange II (AO) solution dissolved in deionized (D.I.) water with pH 3, set by hydrochloric acid. After shaking for 12 h at 37 °C, samples were washed (3 times for 15 min) with hydrochloric acid solution (pH = 3). Samples were shaken for 15 min at 37 °C in NaOH solution (pH = 12) to dissolve the adsorbed AO on the surfaces of the samples. The AO concentration (which was similar to the surface

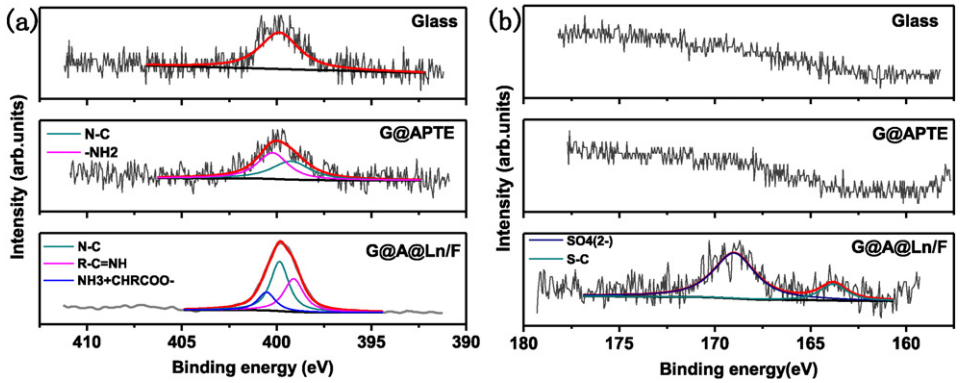

Fig. 2. XPS analysis (a) N1s; (b) s2p high-resolution spectra of Glass, G@APTE and G@A@Ln/F.