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Heparin-loaded zein microsphere film and hemocompatibility

Hua-Jie Wang[a], Zhi-Xin Lin[a], Xin-Ming Liu[b], Shi-Yan Sheng[a], Jin-Ye Wang[a,b,*]

[a]*School of Life Science and Biotechnology, Shanghai Jiaotong University, 1954 Huashan Road, Shanghai 200030, China*
[b]*Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China*

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Abstract

Zein was studied as a drug-eluting coating film composed of zein microspheres for cardiovascular devices (e.g. stent). In vitro 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) analysis showed that both zein film and its degraded product had better biocompatibility compared with Corning culture plate on the growth of human umbilical veins endothelial cells (HUVECs, $p<0.05$, $n=6$), and the effect of zein degraded product on HUVECs was dose-dependent. The best result was obtained at 0.3 mg/ml of the addition. The encapsulation efficiency of heparin and heparin loading varied with the amount of both zein and heparin, and the highest encapsulation efficiency (heparin 1.33 mg/ml and zein 16 mg/ml) was $22.77 \pm 1.33\%$ ($n=3$). Scanning electron microscope (SEM) observation indicated that the zein film was made of microspheres in diameter from nano- to micrometer, which could be controlled. Sizes of heparin-loaded zein microspheres changed before and after release of heparin because of conglutination among zein microspheres. Release rate of heparin from microsphere film reached to $33.5 \pm 1.2\%$ within 12 h, and began to get into subsequent "slow release" phase; about 55% of the entrapped heparin was released after 20 days. Both zein film and heparin-loaded zein microsphere film were effective in suppressing platelet adhesion, and the heparin-loaded film showed a better anticoagulation as determined with thrombin time (TT) assay. These results suggest that zein film could be used directly as a new type of coating material for its better biocompatibility with HUVECs. Moreover, the heparin-loaded zein microsphere film can significantly improve the hemocompatibility.
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Keywords: Film; Hemocompatibility; Microspheres; Platelet adhesion; Zein

1. Introduction

The major drawback of angioplasty is restenosis, which occurs in 30–60% of treated vessels, resulting in the need for repeat procedures [1]. As a cardiovascular device, the potential of stenting technique to reduce the morbidity of restenosis and cost of care is enormous; however, the rate of in-

Abbreviations: HUVECs, human umbilical veins endothelial cells; ECGS, endothelial cells growth supplement; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SEM, scanning electron microscope; TT, thrombin time; PBS, phosphate-buffered solution; FCS, fetal calf serum; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

* Corresponding author. Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China. Tel.: +86 21 54925330; fax: +86 21 64376697.
E-mail address: jywang@mail.sioc.ac.cn (J.-Y. Wang).

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stent restenosis still gets to 10–40% due to unsatisfactory biocompatibility of stents [2]. Up to date, there have been four methods used to solve this problem. The first method is to administer anticoagulant to the patient through injection or oral administration for days, which successfully decreases the rate of the thrombus formation, while the rate of massive hemorrhage is increased. And when the anticoagulant is taken orally, it would be denatured in the digestive tract if the suitable carrier were unavailable [3]. The second method is to modify the surface of the stents with polymers, such as the use of phospholipids [4], which could improve the biocompatibility, especially the blood compatibility. The third method is to coat anticoagulant on the surface of the stents [5], but the concentration of the anticoagulant is difficult to be maintained because of the effect of the blood flow. The fourth method is to seed endothelial cells on the surface of the stents with autogenous endothelium, which could avoid contacting the blood with the material, and therefore suppresses the activation of coagulation factors [6]. Among these methods, drug-eluting stents are called "revolution" in stenting technique, while coated stents have showed huge promise and great efforts have been made to develop the suitable drug and coating materials of stents, which can maintain the drug concentrations at the site of stents deployment and minimize systemic side effects [7]. These drugs used contain heparin, dexamethasone, sirolimus, everolimus, tacrolimus, carvedilol, paclitaxel, actinomycin-D, flavopiridol, angiopeptin and so on [8,9]. These drugs are often blended with synthetic polymers that act as drug reservoirs to elute the active agent over a period of several weeks or months [10]. Unfortunately, many of the synthetic polymers induce an exaggerated inflammatory response and neointimal hyperplasia in animal models [11,12]. It is noted that most of them are nonbiodegradable, and have to be taken out by surgery, which give patients great discomfort. Therefore, the research focuses on searching a slow eluting material with better biocompatibility and biodegradability. The anticoagulant is encapsulated by or combined with the carrier; the compound of drug and carrier can deliver the drug at a dose low enough to avoid systemic effect but high enough to provide local protection against vascular disease [13].

Zein is the major storage protein of corn and accounts for 40–50% of the protein; it is soluble in aqueous alcohol solutions (60–95%). Zein has been used widely, such as adhesive, biodegradable plastics, chewing gum, coating for food products, fiber, cosmetic powder, microencapsulated pesticides and inks. Especially zein has been prepared as microspheres to deliver insulin [14,15]. Our previous work has indicated that zein film, composed of particles in diameter of 100–2500 nm, has good biocompatibilities with both human liver cells and mice fibroblast cells [16]. In present study, zein was considered as a new type of coating material either directly or as a heparin carrier applying for cardiovascular devices such as stent. The biocompatibility of zein film and its degraded product digested with pepsin on human umbilical veins endothelial cells (HUVECs) was studied, the methods of preparing zein microspheres and heparin-loaded microsphere film were investigated, the encapsulation efficiency and release kinetics of heparin-loaded zein microspheres, the morphology of the films, and hemocompatibility (anticoagulation, protein adsorption and platelet adhesion) were tested.

2. Materials and methods

2.1. Materials

Zein with biochemical purity was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), endothelial cells growth supplement (ECGS) was purchased from Upstate Biotechnologies Inc. (Lake Placid, NY, USA), heparin and thrombin were from Sigma (St. Louis, MO, USA), and other reagents were reagent grade.

2.2. Preparation of zein film and its degraded product

Zein was dissolved in aqueous mixture of ethanol, and the ethanol content was brought to 40% immediately to form zein microspheres suspended solution, then the film was prepared through volatilization at 37 °C. The film was sterilized by UV for at least 1 h and immersed into the RPMI 1640 nutrient fluid before use. Zein degraded product was obtained from zein powder digested with pepsin (zein/pepsin = 10:1 w/w)

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