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Covalent attachment of proteins to functionalized polypyrrole-coated metallic surfaces for improved biocompatibility

Wahid Khan, Mamta Kapoor, Neeraj Kumar *

Department of Pharmaceutics, National Institute of Pharmaceutical Education & Research (NIPER), Sector 67, S.A.S. Nagar, 160062 Punjab, India

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Abstract

Stainless steel has been widely used as an implant material for various biomedical applications, but its biocompatibility is still a major issue. Though polymer coating is one of the solutions, biomolecule-attached polymer coating is a better alternative. In this paper, we have synthesized a biomolecule (bovine serum albumin, BSA)-derivatized polymer coating on a stainless steel (316 L) surface and evaluated it for biocompatibility. The monomer used for coating was obtained by hydrolyzing 1-(2-cyanoethyl) pyrrole to 1-(2-carboxyethyl) pyrrole followed by activation with *N*-hydroxysuccinimide to *N*-succinimidyl ester pyrrole. This monomer was electrocoated onto steel plate to provide a smooth and adherent coating of polypyrrole-*N*-succinimidyl ester (PPyNSE) which was characterized in terms of surface morphology and chemical composition by scanning electron microscopy and infrared spectroscopy, respectively. Further, BSA was covalently attached to PPyNSE to obtain a biomolecule-derivatized polymer coating. This coating was evaluated for biocompatibility in terms of thrombus formation, platelet adhesion and hemolysis, and was found to be more biocompatible on these parameters than the bare metal and polypyrrole-coated surfaces. Stability studies on these coated plates were also performed.

Keywords: Polypyrrole (PPy); Implanted medical devices; Biocompatibility; Hemocompatibility; Protein

1. Introduction

Global spending for implantable medical devices was about US$160 billion in 2006. The majority of biomaterials for medical applications are based on metals such as stainless steel and titanium. These materials offer desirable properties, including high tensile strength and stability for medical use [1]; however, their biocompatibility [2] is still a problem that needs to be addressed.

Corrosion resistance and superior mechanical properties make stainless steel one of the most potential candidates for manufacturing implantable medical devices. However, it is not fully biocompatible, but poses various biocompatibility problems. The most commonly encountered biocompatibility problems associated with implantation of metallic medical devices include: (i) inflammatory response; (ii) cell migration and proliferation; (iii) coagulation and hemolysis, caused by interaction of the biomaterial with various blood components; and (iv) thrombosis and restenosis as an arterial response to injury. Besides these, protein adsorption on the surface that occurs immediately after implantation [3] is a key determinant of all subsequent unfavorable biological responses, such as platelet activation, adhesion and thrombus formation [4,5].

Considering these problems, there has been a continuous search for more biocompatible options, which include gold, titanium, cobalt–chromium alloys, tantalum alloys, nitinol and various polymers such as polypropylene, polycarbonates and polyurethanes [4]. Another approach is to modify the steel surface with organic molecules, polymers [6] or inorganic coatings. Improved biocompatibility by surface modification involves: (i) changing the surface properties of the implant, such as surface texture, surface charge, surface energy and surface chemistry, thereby altering the nature of interaction at the material–tissue interface; and (ii) coating the material surface with a poly-

* Corresponding author. Tel.: +91 172 2214683; fax: +91 172 2214692.
E-mail address: neeraj@niper.ac.in (N. Kumar).

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mer or biomolecular layer to prevent its recognition as a foreign body or to create a less reactive layer. This latter approach can be achieved either by: (i) forming a passive surface on the biomaterial which elicits little or no immune response; or (ii) creating an active surface which elicits beneficial responses and suppresses unwanted reaction [7].

Various coating techniques, such as dip coating, spray coating and electropolymerization, are available for coating the surface of a metal. The use of conducting polymers [8] as coating materials and electropolymerization [9] as a coating method offers several advantages: (i) it is simple and reproducible; (ii) it can form an integrated, uniform and durable film; (iii) the coating composition can be easily controlled; (iv) it can enable copolymerization of different monomers; and (v) grafting functional substituents and entrapment of biomolecules on surface is also possible. Pyrrole is one of the most widely used monomers for the preparation of electroconductive polymeric coatings. It offers several advantages, such as easy availability, chemical stability and polymerizability. With the aim of improving the processibility, functionality and physical properties of pyrrole, various pyrrole derivatives have been used [10,11]. The ease in preparation of derivatives, its electropolymerization and surface modification with various specific functional groups makes pyrrole suitable for electrocoating metal surfaces and further provides feasibility of immobilization/coupling of biomolecules. Although polypyrrole and its derivatives have already been used in biosensors and are showing highly attractive results in biomedical applications such as coating for electrodes and neural probes, it is yet to be exploited in the coating of medical devices and for the immobilization/coupling of biomolecules.

Immobilization of biomolecules on modified surfaces can be achieved by three methods: (i) entrapping biomolecules in an electropolymerized film formed with a mixture of monomers and biomolecules; however, the denaturation of the molecules is a possible drawback; (ii) electrostatic binding of biomolecules directly onto specific groups generated on the substrate surface, which constitutes a convenient method for immobilizing proteins; however, the major drawback of this method is the strength of interaction, which is dependent on the surrounding solution, and changes in ionic strength and/or pH can cause the release of the attached moiety; and (iii) covalent attachment of the biomolecules to an electropolymerized polymer film carrying pendant-reactive groups such as ($-NH_2$, –COOH). This is the most promising, as growth of the polymer film and immobilization of the biomolecules can be performed under different experimental conditions, and as a result the denaturation problem can be overcame. Further, there is minimum physisorption, therefore reducing the chances of leaching the captured biomolecules [8].

Albumin is the preponderant blood protein in plasma. Due to its thromboresistant ability, the covalent attachment of albumin on implantable devices has a profound influence on the subsequent events in the blood coagulation cascade, like reduced platelet adhesion and aggregation, thereby avoiding subsequent thrombus formation. Albumin adsorption also reduces fibrinogen adsorption on the surface and lowers plasma protein adsorption, and a high ratio of albumin to fibrinogen adsorption on the coated surface results in the almost complete inhibition of platelet adhesion [3,12].

The physical characteristics and chemical composition of the surface of an implantable device, such as electrical charge, surface chemistry and roughness, are the main factors influencing blood cell adherence, as well as the adhesion of proteins and other molecules [12]. Considering these facts, this paper emphasizes the new application of an already existing conducting polymer with certain modifications to demonstrate its promising potential as a coating material on implantable medical devices by electropolymerization and for immobilization/coupling of biomolecules to improve biocompatibility of implantable devices. We report the preparation and characterization of an *N*-succinimidyl ester (NSE)-functionalized polypyrrole-coated surface followed by the covalent attachment of bovine serum albumin (BSA). These protein-attached surfaces were further evaluated for their stability and biocompatibility.

2. Materials and methods

Stainless steel plates (316 L, SS) of medical grade were used after mechanical and chemical pretreatment. They were mechanically polished successively with 200, 600 and 1000 emery paper to remove any oxide layers from the metal surface and subsequently polished with alumina paste, and chemically degreased with tetrachloroethylene by sonicating for 30 min or rinsing with acetone and distilled water before coating. 1-(2-Cyanoethyl) pyrrole, tetrabutylammoniumtetrafluoroborate and *N*-hydroxy succinimide (NHS) were purchased from Aldrich; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was from Fluka, USA; and BSA (Bovine Fraction V) was from s.d. fine-chem Ltd., India. Ultrapure water was obtained using the SG water purification system (Germany). Other chemicals and reagents were purchased from local Indian sources.

2.1. Synthesis of 1-(2-carboxyethyl) pyrrole monomer [i]

1-(2-Carboxyethyl) pyrrole was synthesized according to the procedure described by Maeda et al. with slight modification [9,13–15]. Briefly, 1-(2-cyanoethyl) pyrrole was hydrolyzed to 1-(2-carboxyethyl) pyrrole by the addition of 5 g (3.6 mM) of 1-(2-cyanoethyl) pyrrole to 10 g KOH in 30 ml of double-distilled water (Fig. 1). This mixture was refluxed under an inert nitrogen atmosphere overnight. Completion of the reaction was confirmed by the disappearance of ammonia using litmus paper and by thin-layer chromatography spot analysis. The resulting product was acidified to pH 4 using 8 M HCl at room temperature

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