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SCIENCE DIRECT®
Biomaterials 27 (2006) 691–701
Biomaterials
www.elsevier.com/locate/biomaterials

Fabrication, characterization, and biological assessment of multilayered DNA-coatings for biomaterial purposes

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Received 30 May 2005; accepted 21 June 2005
Available online 1 August 2005

Abstract

This study describes the fabrication of two types of multilayered coatings onto titanium by electrostatic self-assembly (ESA), using deoxyribosenucleic acid (DNA) as the anionic polyelectrolyte and poly-D-lysine (PDL) or poly(allylamine hydrochloride) (PAH) as the cationic polyelectrolyte. Both coatings were characterized using UV-vis spectrophotometry, atomic force microscopy (AFM), X-ray photospectroscopy (XPS), contact angle measurements, Fourier transform infrared spectroscopy (FTIR), and for the amount of DNA immobilized. The mutagenicity of the constituents of the coatings was assessed. Titanium substrates with or without multilayered DNA-coatings were used in cell culture experiments to study cell proliferation, viability, and morphology. Results of UV-vis spectrophotometry, AFM, and contact angle measurements clearly indicated the progressive build-up of the multilayered coatings. Furthermore, AFM and XPS data showed a more uniform build-up and morphology of [PDL/DNA]-coatings compared to [PAH/DNA]-coatings. DNA-immobilization into both coatings was linear, and approximated 3 μg/cm^2 into each double-layer. The surface morphology of both types of multilayered DNA-coatings showed elevations in the nanoscale range. No mutagenic effects of DNA, PDL, or PAH were detected, and cell viability and morphology were not affected by the presence of either type of multilayered DNA-coating. Still, the results of the proliferation assay revealed an increased proliferation of primary rat dermal fibroblasts on both types of multilayered DNA-coatings compared to non-coated controls. The biocompatibility and functionalization of the coatings produced here, will be assessed in subsequent cell culture and animal-implantation studies.

Keywords: AFM; Cell culture; Cell morphology; Cell proliferation; Cell viability; Electrostatic self-assembly; Fibroblast; FTIR; MIT assay; Mutagenicity; Nanotopography; SEM; Surface modification; Titanium

1. Introduction

In implantology, a wide variety of materials are used to generate biomedical devices with satisfactory properties. Although bulk properties of materials largely

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doi:10.1016/j.biomaterials.2005.06.015

determine their suitability for a given application, the biological response is mainly determined at the biomaterial surface, via the interactions with components of the biological surroundings [1,2].

Modifications of the biomaterial surface have been a major research topic in the past decades. In view of this, an intriguing novel coating material for biomaterials is deoxyribonucleic acid (DNA), the repository of (individual-specific) genetic information. Irrespective of its genetic information, the structural properties of DNA give this unique, natural compound potential for use as a biomaterial coating. The specific build-up of the DNA molecule may ensure a versatile use at various implantation sites. The molecular structure of DNA in vertebrate species is homogenous [3,4], and the non- or low-immunogenic properties of DNA (compared to other biological antigens like proteins and sugars) may limit both innate and acquired immune responses [3,5,6]. Additionally, DNA can incorporate other molecules via groove-binding and intercalation [7,8]. This creates opportunities to specifically deliver desired biological mediators in the direct vicinity of the implantation site. Finally, the high phosphate content in DNA might, via the high affinity of phosphate for calcium ions [9,10], beneficially affect the deposition of calcium in the bone formation process. The use of DNA as a functional biomaterial, instead as a carrier for its genetic information, has already been suggested [11,12], and pioneering efforts have resulted in the fabrication of DNA-containing bulk (bio)material [13], which demonstrated to cause no adverse reactions upon subcutaneous implantation in the backs of rats.

The application of DNA as a coating material, however, is hampered by (a) its easy nucleolytic degradation, and (b) its solubility in aqueous solutions. The use of the previously mentioned DNA-containing bulk material [13] resulted in easily detaching coatings on various substrates. Therefore other methods to obtain stable DNA-containing coatings for biomaterial purposes have to be explored. One applicable technique could be electrostatic self-assembly (ESA), also known as layer by layer (LbL) assembly. Via this technique, polyelectrolyte multilayers (PEMs) can be generated through the alternated progressive adsorption of oppositely charged polyelectrolytes via electrostatic interactions [14].

The ESA technique has been applied successfully using DNA as a polyanionic building block [15–22]. Interestingly, electrostatic interactions with positively charged polyelectrolytes have demonstrated to protect DNA from degradation by nucleolytic activity [23]. Furthermore, the method of fabrication (i.e. under immersion in aqueous solutions) inherently evidences the water insolubility of PEMs. Even immersion of PEMs in high ionic solutions (i.e. exceeding those in physiological conditions) does not cause dissociation of the PEM structure. Instead, changes in the ionic content of the polyelectrolyte solutions in the fabrication process are one of the means to modulate PEM properties, including layer thickness [14,24].

Irrespective of the progress made so far, the use of DNA-containing multilayered coatings in the biomaterials field still requires their complete characterization. Whereas the build-up mechanism of PEMs has been described previously, information on the morphological properties is scarce. Additionally, the hypothesized beneficial properties of DNA necessitate the quantification of the amount of DNA immobilized in the coatings. Therefore, the aim of this study was to fabricate and characterize two types of multilayered DNA-coatings, which differ in the type of cationic polyelectrolyte used, and assess their effects on cell behavior using in vitro-experiments.

2. Materials and Methods

2.1. Materials and fabrication

2.1.1. Materials

Polyanionic DNA ($\pm$300 bp/molecule; sodium salt) was kindly provided by Nichiro Corporation (Yokosuka-shi, Kanagawa prefecture, Japan). Potential protein impurities in the DNA were checked using the BCA protein assay (Pierce, Rockford, Illinois, USA) and measured to be below 0.20% w/w (data not shown). Polycationic polyelectrolytes poly(allylamine hydrochloride) (PAH; MW ~70,000) and poly-D-lysine (PDL; MW 30,000–70,000) were purchased from Sigma (Sigma-Aldrich Chemie B.V., Zwijndrecht, the Netherlands). All materials were used without further purification. The monomeric structures of the cationic polyelectrolytes used are presented in Fig. 1.

2.1.2. Substrate preparation and cleaning

Three types of substrates were used:

1. Quartz substrates (45 × 12 mm; Hellma Benelux B.V., Rijswijk, the Netherlands) were used for UV-vis spectrophotometry, contact angle measurements, FTIR spectroscopy, and XPS.
2. Silicon substrates (1 × 1 cm; Wafernet GmbH, Eching, Germany) were coated with a 50 nm thick titanium layer, using the RF magnetron sputter technique. The titanium sputtering process (duration 5 min, pressure ~5×10^{-4} mbar) was performed with the silicon substrates attached to a rotating sample holder. Titanium-sputtered silicon substrates were used for XPS, contact angle measurements, and AFM.
3. Disc-shaped titanium substrates (12 mm diameter, 2 mm thickness; commercially pure titanium, as-machined) were used to determine the incorporation of radiolabeled DNA into each double-layer.