Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 124–133
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

A new approach to graft bioactive polymer on titanium implants: Improvement of MG 63 cell differentiation onto this coating

Gérard Hélary *, Flavie Noirclère, Josselin Mayingi, Véronique Migonney

Laboratoire des Biomatériaux et Polymères de Spécialité, UMR 7032, Université Paris 13, Avenue Jean Baptiste Clément, 93430 Villetaneuse, France

Received 3 March 2008; received in revised form 28 July 2008; accepted 31 July 2008
Available online 28 August 2008

Abstract

Integration of titanium implants into bone is only passive and the resulting fixation is mainly mechanical in nature, with anchorage failure. Our objective, to increase the biointegration of the implant and the bone tissue, could be obtained by grafting a bioactive ionic polymer to the surface of the titanium by a covalent bond. In this paper, we report the grafting of an ionic polymer model poly(sodium styrene sulfonate) (polyNaSS), in a two-step reaction procedure. Treatment of the titanium surface by a mixture of sulfuric acid and hydrogen peroxide allows the formation of titanium hydroxide and titanium peroxide. In the second reaction step, heating of a metal implant, placed in a concentrated solution of sodium styrene sulfonate monomer (NaSS), induces the decomposition of titanium peroxides with the formation of radicals capable of initiating the polymerization of NaSS. Various parameters, such as temperature of polymerization and time of polymerization, were studied in order to optimize the yield of polyNaSS grafting. Colorimetry, Fourier-transformed infrared spectra recorded in an attenuated total reflection, X-ray photoelectron spectroscopy techniques and contact angle measurements were applied to characterize the surfaces. MG63 osteoblastic cell response was studied on polished, oxidized and grafted titanium samples. Cell adhesion, alkaline phosphatase activity and calcium nodules formation were significantly enhanced on grafted titanium samples compared to unmodified surfaces.
© 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

Keywords: Titanium; Surface grafting; Cell differentiation; Bioactive polymer

1. Introduction

Although titanium implants are widely used in orthopaedic fields, insufficient integration into surrounding bone often occurs. An uncontrolled inflammation process inducing fibrous capsule formation prevents the generation of a stable implant to host tissue binding and consequently implants can fail under shear stress, requiring revision surgery [1].

Over the last 10 years, many studies have been devoted to increasing the osteointegration by modifying surface properties (roughness, topography, surface charges, passivation and wettability) using different methods, such as mechanical treatment [2,3], chemical treatment [4], thermal treatment [5,6] and electrochemical methods [7,8]. However, even if modified surfaces have a higher early level of cell attachment than untreated titanium surface, the successful implantation rate is not satisfying.

Biochemical methods of surface modification are promising approaches, at least at the laboratory level [9–11]. The aim is to control the tissue–implant interface by the immobilization of proteins, enzymes or peptides for the purpose of inducing specific cell responses. The most investigated peptide sequence immobilized to titanium or polymer surfaces is RGD (arginine–glycine–aspartic acid) derived from fibronectin and recognized by almost all α/β integrins [12]. The main difficulty is to ensure the stability of the biomolecules binding to the surface of the implant and its accessibility to active sites of cells. Physical adsorption is not successful for long-term implantation mainly due to the desorption of biomolecules. The covalent attachment of

* Corresponding author. Tel.: +33 1 49 40 36 80.
E-mail address: ghelary@galilee.univ-paris13.fr (G. Hélary).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.07.037

biomolecules to the titanium surface allows the problem of disruption under physiological medium or mechanical stress to be resolved. Covalent attachment requires the use of different chemical reactions which can be aggressive towards the biomolecules, reducing their potential bioactivity [13]. Other problems are the cost of the biomolecules, which can be a limitation to industrial applications, as well as the possible in vivo enzymatic degradation.

In our laboratory, we have shown that polymers bearing appropriate chemical functions can modulate the cell attachment and spreading onto these bioactive polymers and the cell activity [14,15]. The distribution of these ionic groups along the macromolecular chains creates active sites which can interact with extracellular proteins, such as fibronectin, implicated in cell response.

In this paper, we describe the grafting of an ionic group such as sulfonate groups by radical polymerization of monomers bearing these chemical functions (sodium styrene sulfonate NaSS). Oxidation of the titanium creates titanium peroxides at the surface which under heating produce radicals initiating the polymerization of ionic monomer NaSS. Various parameters to optimize the grafting of polyNaSS on titanium samples were studied. The influence of the grafted surfaces on MG63 cells adhesion and mineralization is discussed.

2. Materials and methods

2.1. Materials

Titanium foil from Alfa Aesar (purity: 99.7%; 0.5 mm in thickness) was cut into squares 1 cm^2 in size. Sodium styrene sulfonate NaSS, hydrogen peroxide (30 wt.%) were purchased from Fluka. Sodium styrene sulfonate was purified by recrystallization in a mixture of water/ethanol 10/90 vol.%.

2.2. Preparation of titanium samples

Titanium samples were mechanically polished with wetted metallographic polishing (grade 800, 1000 and 1200). These samples were referred to as Ti_{1200}. After polishing, Ti_{1200} samples were cleaned in an ultrasonic bath: 10 min in acetone followed by 20 min in distilled water. Prior to cell culture, all samples were washed with 1.5 M NaCl, 0.15 M NaCl, pure water and phosphate-buffered saline (PBS), three times each, and sterilized by ultraviolet (UV) irradiation under a germicide UV lamp with a power of 30 W applied for 15 min on each titanium face, which is not enough to degrade the polymeric coating.

2.3. Grafting of bioactive polymers on titanium

Ti_{1200} samples were ultrasonically cleaned in the following solvents: hexane, acetone and water, for 15 min each. After drying under vacuum for a few hours, there were kept under argon. Oxidation was performed by immersion in sulphuric acid for 1 min before an identical volume of hydrogen peroxide solution was added. (The details of this procedure will be presented in the Sections 3 and 4.) Then samples were rinsed extensively with water. After drying, samples were immersed in an aqueous solution containing monomers (NaSS) at a concentration of 0.7 M. The solution was heated at a temperature of 70 °C for 15 h. Samples were rinsed three times under stirring with water to remove non-reacted monomers.

2.4. Surface characterization

2.4.1. ATR–FTIR

Fourier-transformed infrared (FTIR) spectra, recorded in an attenuated total reflection (ATR), were obtained using a Thermo Nicolet Avatar 370 Spectrometer. Spectra were obtained with a 4 cm^{-1} resolution using a 45° Ge crystal. Samples were pressed against the crystal using a smart Omni sampler. Data presented are averaged from 128 spectra.

2.4.2. Colorimetric method

Complexation of quaternary ammonium groups of toluidine blue with sulfonate groups allows the amount of NaSS grafted to titanium samples to be determined. According to Ikada et al. [16], the toluidine blue is capable of making a complex through its $N^+(CH_3)_2$ with the sample's carboxylate. This concept was transposed to the sodium styrene sulfonate by preparing a network of polyNaSS which was immersed in a solution of toluidine blue. After rinsing, each sulfonate group was complexed with the $N^+(CH_3)_2$ of the marker. Grafted samples were immersed in an aqueous solution of toluidine blue (5×10^{-4} M) for 6 h at 30 °C. Then samples were rinsed with an aqueous solution of NaOH (5×10^{-3} M) in order to remove uncomplexed dye. A standard series was done with seven different concentrations between 4×10^{-6} and 5×10^{-5} M, which allows us to determine the concentration of decomplexed toluidine blue thanks to the molar extinction coefficient. Decomplexation of toluidine blue occurs by immersing titanium samples in an aqueous solution of acetic acid (50 vol.%) for 24 h. Concentration of decomplexed toluidine blue is measured by visible spectroscopy at 633 nm using a Perkin-Elmer spectrometer lambda 25.

2.4.3. XPS

X-ray photoelectron spectroscopy (XPS) analyses were conducted using an Escalab VG 220i-XL spectrometer (VG instruments). X-rays were produced by a monochromatic Al K_α source of 1486.6 eV. The photoelectron takeoff angle (the angle between the sample normal and the input axis of the energy analyzer) for all XPS experiments was 45°. This takeoff angle corresponds to a sampling depth of approximately 10 nm. The energy resolution was 0.1 eV. Four spots were analyzed for each sample.

The Ellipse program from VG was used to determine peak areas, calculate the elemental compositions from

ID	Title	Pages
2352	A new approach to graft bioactive polymer on titanium implants: Improvement of MG 63 cell differentiation onto this coating	10

Download Full-Text Now

http://fulltext.study/article/2352

fullText.study

Categorized Journals
Thousands of scientific journals broken down into different categories to simplify your search

Full-Text Access
The full-text version of all the articles are available for you to purchase at the lowest price

Free Downloadable Articles
In each journal some of the articles are available to download for free

Free PDF Preview
A preview of the first 2 pages of each article is available for you to download for free

http://FullText.Study