Applied Surface Science 355 (2015) 553–561

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

New nanostructured nickel–polymer nanohybrids with improved surface hydrophobicity and effect on the living cells adhesion

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ARTICLE INFO

Article history:
Received 17 February 2015
Received in revised form 26 June 2015
Accepted 17 July 2015
Available online 18 July 2015

Keywords:
Cell adhesion
Nanostructured surfaces
Nickel nanostructures
Polymer thin film

ABSTRACT

An intensive gain of surface hydrophobicity has been observed on the differently polar polymer layers spin-coated directly on the previously prepared nanostructured nickel surface to form nanohybrids. Nanostructured nickel layer has been prepared by electrochemical deposition to form polyhedral crystalline nanostructure. Surface morphology and homogeneity of a nanohybrid polymer layer have been monitored by TOF-SIMS and SEM methods. Hydrophobicity extension of nanohybrid surfaces increased nearly linearly with decreasing polarity of single polymers applied and maximum increase in hydrophobicity value obtained was 32%. Novel nanohybrid surfaces functionality has been tested on the different cells adhesion. The results showed cell adhesion followed with an inhibition of the living cells spreading and proliferation on declared nanostructured nickel–polymer nanohybrid surfaces. The maximum inhibition activity of nanohybrid surface against cells line has been observed in a case when polydimethylsiloxane was applied as surface polymeric layer. Preparation of this kind of surface is easy and inexpensive, with many proposed applications where hydrophobic surfaces are required. This also can tend as a model for the preparation of the surfaces with cell anti-adhesion and antimicrobial activity.

1. Introduction

The research of functional nanoscale hybrid materials is one of the most promising and rapidly emerging research areas in materials chemistry. Nanoscale hybrid materials can be broadly defined as synthetic materials with organic and inorganic components which are linked at nanometre scale. This approach allows creating a number of novel advanced materials with well-controlled structures and multiple functions. The unique properties of advanced hybrid nanomaterials can be favourable to many fields such as medicine (antibacterial surfaces for instruments, implant surfaces, etc.), self-cleaning surfaces, and a route to new materials. The features of the surface where the cells are attached can significantly control their behaviour [1]. This way could be controlled processes such as cell proliferation [2–5], migration [6] and apoptosis [7]. The features on nanostructured surface can also induce the change of cell shape [3,6]. This phenomenon could be used to modulate and manage cells to reach exact results. Recent status in a research field represents the articles where metal nanoparticles were dispersed in a polymer matrix with resulting functional properties [8] or dispersing hydrophilic nanoparticles in hydrophobic polymers [9]. The anti-biofilm properties of silver and gold incorporated different polymers with polymethylmetacrylate (PMMA) have been also well documented [10]. Composites reduced growth of the organism and they may be suitable for implant applications. One of the desired results is to support adhesion of the cells on the surface of bone implants [11–14] and prosthesis. Metallic bone implants with surface of anodic alumina oxide (AAO) possess great chemical and mechanical stability and have regular self-organized orthogonal surface with pore size ranging between 5 nm and 10 μm [15]. Metallic nanostructured surfaces prepared by elechrochemical deposition are of low cost, flexibility

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http://dx.doi.org/10.1016/j.apsusc.2015.07.104
0169-4332/

and undemanding experiment set-up. It is considered as an inexpensive and versatile method for preparing thin films, and unlike most alternatives, it does not require a vacuum system. Electrodeposited films can be produced by controlling either the current density or deposition potential, the former being known as galvanostatic control while the latter is known as potentiostatic control. Despite the simplicity in experimental set-up and low cost, the phenomenon of electrodeposition is very complex, since it is typically a non-equilibrium growth process and it involves so many variables, such as electrolyte pH and concentration, cation diffusion, and type of substrate. Films grown under non-equilibrium conditions are expected to develop self-affine surfaces [16], whose roughness varies as a function of film thickness. Surface and interface roughness are important parameters affecting the physical properties of multilayers and sandwich structures [17,18]. Nanostructured functionalities on surfaces have also potential in the diagnosis of cancer diseases in early stage [19,20] and blocking cancer cells growth [15]. In general, the cell adhesion is influenced by a lot of factors but the surface properties, such as wettability, roughness, surface charge, chemical functionalities, stiffness and interactions of scaffold degradation products [21] play the dominant role. In the biological applications, the wettability of nanostructured surface is considered to be the most important attribute of the nanostructured surfaces influencing cell adhesion [7] and biological response on artificial materials [21]. It has been shown that cells achieve maximum attachment onto surfaces with moderate wettability [3,7]. Increasing of wettability of hydrophobic surfaces leads to increased adsorption of proteins and stronger cell adhesion [14,22]. Low surface wettability can also inhibit adhesion of bacteria cells. Yao et al. prepared nanostructured polyurethane surface with enhanced hydrophobicity and greater water contact angle to prevent bacteria adhesion onto surface of medical devices [23]. Sotriou et al. prepared nanostructured surface consisting of silver nanoparticles immobilized onto SiO_2 substrate [24]. Similar substrate made of silver nanoparticles attached onto TiO_2 substrate of bone implant was prepared by Ferraris et al. [25]. Both of previously mentioned silver surfaces have antibacterial effect. Cells adhesion is an extremely complicated process that is affected by many factors, including environmental issues, the associated flow conditions, the presence of serum proteins or antibiotics, the cell or bacterial properties, and the material surface characteristics [26,27]. Bacterial adhesion to a material surface can be described as a two-phase process, including an initial, instantaneous, and reversible physical phase (phase one), followed by a time-dependent and irreversible molecular and cellular phase (phase two) [26–28]. Phase one of the bacterial adhesion consists in the initial attraction of the cells to the surface through the effects of physical forces, such as Brownian motion, van der Waals attraction forces, gravitational forces, the effect of surface electrostatic charge, and hydrophobic interactions [27–29]. These physical interactions are further classified as long-range interactions (nonspecific, distances > 50 nm between cells and surfaces) and short-range interactions (distances < 5 nm, with involvement of hydrogen bonding, ionic, and dipole interactions and hydrophobic interactions). Now it is clearly shown how nanosized dimension plays role in nanostructured surface effect on cell adhesion. Long and short interactions are fundamental for the initial part of cells adhesion to surfaces, which makes the molecular or cellular phase of adhesion possible [26–28,30]. The cells properties (cells and bacterial hydrophobicity, cells surface charge) and the material surface characteristics (surface chemical composition, surface roughness, and surface configuration) are important in bacterial adhesion to uncoated surfaces and can potentially be targeted in antiadhesion therapy [29,31,32]. Phase two consists in molecular specific reactions between bacterial surface structures and substratum surfaces, uncoated or coated with host matrix proteins (i.e., albumin, fibronectin, fibrinogen, vitronectin, and laminin). Biological cell adhesion is regulated by adhesion promoting and adhesion inhibiting factors. Promoting factors are integrins, focal adhesion kinase (FAK) and Src kinases, between adhesion inhibiting factors belong SIRP inhibitory receptor activated by interacting with CD47 extracellular ligand and SHP-1. Vimentin is also important in cell strengthening of cell adhesion [26]. Cells are attached (if adhered) to surface via focal adhesion points. Before focal adhesion is made, the α and β chain of integrin is activated and change conformation is done by bonding to surrounding environment. After bonding integrin–ligand integrins cluster and give an upraise to focal adhesions [33–35]. Cells interact with extracellular matrices primarily through integrins, a widely expressed family of cell surface receptors, and integrin binding to its extracellular ligand is responsible for the downstream effects of the matrix on cell function. For example, in the muscle differentiation system, antibodies to b1 integrin reversibly block differentiation and retain cells in a proliferating state. This fundamental principle of regulation of developmental phenotype through binding of integrin receptors has been demonstrated for a variety of other systems, including mammary and kidney epithelial cells and keratinocytes. This interaction is governed by the surface densities of integrin receptors and their ligands and the receptor–ligand binding affinities. Integrin receptors undergo changes in conformation in response to intracellular signals that are capable of modulating their ligand binding affinity. This modulation of integrin binding has been shown to play roles in epithelial and muscle differentiation. Cell adhesion onto different surfaces results in conformational changes that lead to differences in integrin receptor binding and modulate the switch between cell proliferation and differentiation.

This paper deals with a new functionality finding of an intensive extension in the surface hydrophobicity when differently polar polymers (biocompatible) were spin-coated directly on nanostructured nickel surface to form the nanohybrids. Unique strong surface hydrophobicity increase is due to an extreme surface improvement by nanostructured nickel that was copied by spin-coated polymer layer. The adhesion of the different living cells on various nanohybrid surfaces has been studied. Unique nanostructured nickel–polymer nanohybrid materials open the way to further valorisations of a ubiquitous antibacterial surface, cell growth control as well as renewable resource in applications such as water repellence and self-cleaning.

2. Experimental

All operations were performed at atmospheric pressure and temperature of 25 °C, except of cell culturing process (37 °C) and their fixation with paraformaldehyde which was performed at 4 °C. Circular stainless steel plates were cut from 316L stainless steel (Alfa Aesar, Germany) with diameter of 24.6 mm to reach best fit into spin-coater. Used chemicals were p.a. grade.

$NiSO_4{\cdot}7H_2O$, $NiCl_2{\cdot}6H_2O$, sodium citrate, H_3BO_3 and paraformaldehyde were purchased from Alfa Aesar GmbH (Germany). DMEM, DPBS, FBS and Hog Trypsin were purchased from Biological Industries (Czech Republic). Penicillin/Streptomycin PreMix were purchased from Carl Roth GmbH + Co KG (Germany). RPMI-1640 medium was supplemented with 10% FBS and antibiotics (penicillin 100 U mL^{-1}, streptomycin 100 μg mL^{-1}, amphotericin 25 μg mL^{-1}), all from Gibco (Invitrogen, Grand Island, NY, USA). All used solutions were freshly prepared. In experiments applied silicone polymers: (polydimethylsiloxane (PDMS), cyanopropylmethylphenylmethylsilicone (CNPhMS) and phenyl (50%) methylsilicone (PhMS)) were purchased from Applied Science Laboratories Inc. (USA). The polarities for all used polymers are given in Table 1.