Colloids and Surfaces B: Biointerfaces 157 (2017) 309–316

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Protocols

Cell-compatible conducting polyaniline films prepared in colloidal dispersion mode

Věra Kašpárková[a,b], Petr Humpolíček[a,c,*], Zdenka Capáková[a], Patrycja Bober[d], Jaroslav Stejskal[d], Miroslava Trchová[d], Petra Rejmontová[a,c], Ita Junkar[e], Marián Lehocký[a], Miran Mozetič[e]

[a] Centre of Polymer Systems, Tomas Bata University in Zlin, 76001 Zlin, Czech Republic
[b] Department of Fat, Surfactant, and Cosmetics Technology, Faculty of Technology, Tomas Bata University in Zlin, 760 01 Zlin, Czech Republic
[c] Polymer Centre, Faculty of Technology, Tomas Bata University in Zlin, 760 01 Zlin, Czech Republic
[d] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague 6, Czech Republic
[e] Department of Surface Engineering, Plasma Laboratory, Josef Stefan Institute, 1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form 20 May 2017
Accepted 26 May 2017
Available online 31 May 2017

Keywords:
Polyaniline
Conducting films
Colloidal dispersions
Surface analysis
Cell compatibility
Skin irritation

ABSTRACT

Conducting polyaniline can be prepared and modified using several procedures, all of which can significantly influence its applicability in different fields of biomedicine or biotechnology. The modifications of surface properties are crucial with respect to the possible applications of this polymer in tissue engineering or as biosensors. Innovative technique for preparing polyaniline films *via in-situ* polymerization in colloidal dispersion mode using four stabilizers (poly-*N*-vinylpyrrolidone; sodium dodecylsulfate; Tween 20 and Pluronic F108) was developed. The surface energy, conductivity, spectroscopic features, and cell compatibility of thin polyaniline films were determined using contact-angle measurement, the van der Pauw method, Fourier-transform infrared spectroscopy, and assay conducted on mouse fibroblasts, respectively. The stabilizers significantly influenced not only the surface and electrical properties of the films but also their cell compatibility. Sodium dodecylsulfate seems preferentially to combine both the high conductivity and good cell compatibility. Moreover, the films with sodium dodecylsulfate were non-irritant for skin, which was confirmed by their *in-vitro* exposure to the 3D-reconstructed human tissue model.

1. Introduction

The combination of intrinsic electrical conductivity with easy preparation and modification procedures opens the door for the promising application of conducting polymers in engineering of excitable tissues. Among conducting polymers, polyaniline (PANI) receives particular and still increasing attention, mainly because it can be prepared by various methods [1–3] and it is subsequently easily modified in order to change its surface properties [4]. Therefore, this polymer seems to be well suited for application in tissue engineering and biosensors. PANI prepared in powder form, however, possesses notable cytotoxicity [5], which is believed to be mainly connected to the presence of low-molecular-weight impurities [6]. The purification of PANI powder after preparation [7], as well as methods of its green synthesis [8], are alternatives to the standard route of preparation [2]. Compared to powders, thin PANI films grown on the supporting surfaces seem to be purified more easily. Thanks to this fact, pristine PANI films [9,10] as well as films combined with other polymers [11] enable cell growth on their surfaces. PANI films coated with poly(2-acrylamido-2-methyl-1-propanesulfonic acid) were compatible with blood cells and allowed for platelet adhesion [12]. Another way how cell compatibility, or more generally the biocompatibility of PANI, can be controlled is the combination with various stabilizers, whether they are standard surfactants or water-soluble polymers. Some of these stabilizers have been used to prepare colloidal PANI [13]; some were even tested for their biological properties [14]. In comparison with standard PANI films, they have reduced surface roughness, as the stabilizer prevents the macroscopic precipitation of PANI from the reaction mixture. Moreover, films grown on surfaces immersed in mixtures containing stabilizers are thinner compared to those prepared from a reaction mixture without stabilizer [15]. The present study therefore focuses on PANI

* Corresponding author at: Centre of Polymer Systems, Tomas Bata University in Zlin, Trida Tomase Bati 5678, 76001 Zlin, Czech Republic.
E-mail address: humpolicek@utb.cz (P. Humpolíček).

http://dx.doi.org/10.1016/j.colsurfb.2017.05.066
0927-7765/

modification using various surfactants or colloidal stabilizers with the aim to control the surface and electrical properties and also the cell compatibility.

2. Materials and methods

2.1. Preparation of polyaniline films

PANI films were prepared *in situ* on a suitable substrate *via* the oxidation of aniline hydrochloride (0.2 mol L^{-1}, Lach-Ner) with ammonium peroxydisulfate (0.25 mol L^{-1}, Sigma-Aldrich) in the presence of a stabilizer (2 wt%). Four different substrates were used as supports: tissue polystyrene culture dishes (TPP; Switzerland) for the determination of cell compatibility, a glass support for conductivity measurements, polypropylene foil for the measurement of surface properties, and silicon wafer for spectroscopic analysis. To produce PANI films, aniline hydrochloride (2.59 g) was dissolved in 50 mL of an aqueous solution of the respective stabilizer (40 g L^{-1}), namely poly(*N*-vinylpyrrolidone) (PVP), sodium dodecylsulfate (SDS), Tween 20 (T20), or Pluronic F108 (F108) (all Sigma-Aldrich). An aqueous solution (50 mL) containing 5.71 g of ammonium peroxydisulfate was added, and the reaction mixture was stirred and poured over the substrate. The oxidation of aniline was left to proceed for 1 h. PANI films formed on supports were then rinsed with 0.2 mol L^{-1} HCl and left to dry in air. The samples were designated PANI-PVP-H_2O, PANI-SDS-H_2O, PANI-T20-H_2O, and PANI-F108-H_2O. Similar films were also prepared with aniline hydrochloride and stabilizer dissolved in 1 mol L^{-1} hydrochloric acid instead of water (samples PANI-PVP-HCl, PANI-SDS-HCl, PANI-T20-HCl, and PANI-F108-HCl), *i.e.* under higher acidity of the reaction medium. The films prepared in the absence of stabilizer are referred to as standard films.

2.2. Conductivity

The conductivity of the samples was measured by the four-point van der Pauw method. A programmable electrometer with an SMU Keithley 237 current source and a Multimeter Keithley 2010 voltmeter with a 2000 SCAN 10-channel scanner card were employed. Measurements were carried out at ambient temperature.

2.3. Surface energy

Contact angle measurements and the determination of surface energy were conducted with the aid of the "SEE system" (surface energy evaluation system) (Advex Instruments, Czech Republic). For polyaniline samples, deionized water, ethylene glycol, and diiodomethane (Sigma–Aldrich) have been used as test liquids. The droplet volume of the test liquids was set to 2 μL in all experiments. Ten separate readings were averaged to obtain one representative contact-angle value. By using these data, the substrate surface free energy was determined by the "acid–base" method.

In order to evaluate the interaction of fibroblasts cells with PANI films, the surface free energy of the cells was also determined. The cell suspension was thoroughly filtered through a filtration paper to obtain a homogeneous cell layer on the paper, and this sample was immediately subjected to contact-angle measurement. For cells, glycerol (Sigma–Aldrich) and diiodomethane were used as the test liquids, and the droplet volume of these liquids was also set to 2 μL for all experiments. Correspondingly to PANI films, ten separate readings were taken to obtain one representative average contact angle value. Using these data, the cell surface free energy was calculated by the Owens-Wendt-Rabel-Kaelble (OWRK) method, and the total surface energy (γ^{tot}), including its components, the disperse part (γ^{LW}) and the polar part (γ^{AB}), were obtained. Finally, the cell-film interaction was determined and γ^{dif}, denoting the absolute value of the difference between the surface energy of cells and that of the film, was calculated according to the equation $\gamma^{dif} = |\gamma^{tot,cell} - \gamma^{tot,sample}|$.

2.4. Spectroscopic analysis

Fourier-transform infrared (FTIR) spectra of the films deposited on silicon windows were recorded in the range of 650–4000 cm^{-1} at 256 scans per spectrum at a 4 cm^{-1} resolution by means of a fully computerized Thermo Nicolet NEXUS 870 FTIR Spectrometer using a DTGS detector. After measurements, spectra were corrected for moisture and carbon dioxide in the optical path. An absorption subtraction technique was used to remove the spectral features of silicon wafers. Golden GateTM Heated Diamond ATR Top-Plate (MKII Golden Gate single reaction ATR system) was applied for the supporting measurements of pure surfactants.

Raman spectra excited with an HeNe 633 nm laser were collected on a Renishaw inVia Reflex Raman microspectrometer. A research-grade Leica DM LM microscope with ×50 objective magnification was used to focus the laser beam on the sample placed on an X–Y motorized sample stage. The scattered light was analyzed by a spectroscope with holographic gratings with 1800 lines mm^{-1}. A Peltier-cooled CCD detector (576 × 384 pixels) was used to register the dispersed light.

2.5. Cell compatibility

Prior to *in-vitro* testing, the samples were disinfected by 30 min exposure to a UV-radiation source operating at a wavelength of 258 nm emitted from a low-pressure mercury lamp. The compatibility of polyaniline films with the mouse embryonic fibroblast NIH/3T3 cell line (ATCC; US) was investigated. Cell adhesion, proliferation, and migration were studied. The ATCC–formulated Dulbecco's Modified Eagle's Medium (PAA; Switzerland) containing 10% calf serum (PAA; Switzerland) and 100 U mL^{-1} Penicillin/Streptomycin (PAA; Switzerland) was used as the culture medium.

Tests were conducted as follows: (1) To reveal the ability of cells to adhere to surfaces, the cells were seeded on reference culture dishes (TPP; Switzerland) and the studied polymer films in a concentration of 1×10^7 cells mL^{-1}. After one or six hours, the cells were gently rinsed and micrographs were taken. (2) Cell proliferation was evaluated with cells seeded in an initial concentration of 1×10^5 cells mL^{-1} and cultivated for 72 h. Micrographs were also taken. (3) To show the changes in cytoskeleton, the actin filaments staining with ActinRed™ 555 (Thermo Fisher Scientific, USA) was used. Cells were seeded in an initial concentration of 1×10^5 cells mL^{-1} and cultivated for 24 h. Subsequently, cells were fixed and permeabilized using the following procedure. Cells were fixed using 4% formaldehyde (Penta, Czech Republic) for 15 min, rinsed with PBS and subsequently poured with 0.5% Triton X-100 (Sigma-Aldrich,USA) for 5 min for permeabilization. After this time, cells were rinsed 3 times with PBS (Invitrogen, USA). PBS (1 mL) containing 10 μL of ActinRed™ 555 was added and cells were left to incubate for 30 min in the dark. Morphology of cells was observed and micrographs were taken using an inverted fluorescence phase contrast microscope (Olympus IX 81, Japan). (4) Cell migration was determined by scratch assay according to Liang et al. [16] with modification. A scratch was created in a confluent cell monolayer and after 24 h micrographs were captured with an Olympus inverted fluorescent microscope (Olympus, IX 51; Japan) equipped with a digital colour camera (Leica DFC480; Germany).