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Oxygen and ammonia plasma treatment of poly(3-hydroxybutyrate) films for controlled surface zeta potential and improved cell compatibility

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ABSTRACT

The oxygen and ammonia radio-frequency (RF) plasma treatment of poly(3-hydroxybutyrate) P3HB films was performed. We revealed significant changes in the topography, a decrease in the surface zeta potential from −63 to −75 mV after the oxygen–plasma treatment and an increase after ammonia plasma treatment from −63 to −45 mV at a pH of 7.4. Investigations into the NIH 3T3 fibroblast adhesion and growth demonstrated the best cell vitality and a higher cell number for the ammonia plasma treatment at 150 W.

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1. Introduction

Degradable polymers are prospective materials for tissue engineering due to their biocompatibility, biodegradation and wide range of physical and mechanical characteristics [1]. Poly(3-hydroxybutyrate) (P3HB) is a well-studied polyhydroxyalkanoate (PHA) [2]. It is used for surgical sutures, stents, orthopaedic pins, articular cartilage repair devices, tendon repair devices, nerve repair devices, cardiovascular patches, and wound dressings. However, the application of P3HB is limited due to the hydrophobic character of its surface [3]. One approach to improve the surface wettability and biological compatibility of P3HB involves a surface modification that promotes interaction with the surrounding tissues. Plasma modification enables gentle surface processing without significant destruction of the polymer chains. Plasma processing promotes the formation of new chemical functional groups that change the surface charge of polymers without altering their physical and mechanical characteristics.

The most commonly used methods to immobilize different bioactive compounds on polymer surfaces are based on adsorption using electrostatic interactions [4]. The surface charge of a polymer film depends on its chemical composition. A suitable surface zeta potential promotes the adhesion and proliferation of fibroblasts and other cell types [5,6]. Therefore, this study discusses the correlation between different plasma treatment conditions of P3HB and the surface topography, zeta potential, cell attachment and proliferation.

2. Experimental procedures

Polymer thin films were formed by casting a solution of homopolymer PHB (weight average molecular weight Mw 9.6×10^5 Da, polydispersity Đ 5.3) in chloroform (40 ml of 2% solution (m/v)) on degreased glass (Petri dishes) and then drying the solution at room temperature. As a result of the preparation, thin films of P3HB in the form of disks of approximately 10 cm in diameter and 60–70 μm in thickness were obtained.

The films were modified using an atmosphere of either pure (99.99 %) O_2 or NH_3 gas in an RF plasma discharge (13.56 MHz) at a power level of either 100 or 150 W. The treatment time was 20 min for all experiments.

The surface topography was obtained using atomic force

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microscopy (AFM, SOLVER) (NT-MDT, Russia) in non-contact mode. The zeta potential was analyzed by measuring the streaming potential for the P3HB films at pH values from 6 to 8 using the SURPASS (Anton Paar) system. A KCl solution with a 0.001 M concentration was used as the electrolyte. The pH was changed from the initial value of ~6 using a step-wise addition of small amounts of a dilute KOH solution. The zeta potential was determined from the streaming potential at various flow rates [7].

For the cell proliferation studies, polymer films of 15 mm in diameter were used. The disks were sterilized using 70% ethanol for 10 min followed by subsequent UV-irradiation. The UV irradiation conditions were adjusted to avoid any changes in the surface chemistry.

Sterilized P3HB disks were introduced into a 24-well tissue culture plate (Techno Plastic Products AG). NIH 3T3 fibroblast cells (Institute of Cytology and Genetics of SB RAS) were seeded onto the P3HB films at a density of 10^5 cells per cm^2 to evaluate their attachment, proliferation and growth. The experiments were performed in Dulbecco's Modified Eagle Medium (DMEM) with the addition of 10% calf serum and an antibiotic solution (streptomycin 100 μg/ml, penicillin 100 ME/ml) (Sigma) in a 5% CO_2 atmosphere at 37 °C for 7 days. The investigation of the cell adhesion was performed using the results of the cell morphology based on the SEM images (TM 3000, Hitachi, Japan) and fluorescent staining (DAPI (4',6-diamidino-2-phenylindole) and FITC (fluorescein isothiocyanate)). The images were taken at a voltage of 5 and 15 kV. A DAPI solution with a concentration of 300 nM was used. After fixation, the cells were washed three times in Dulbecco's phosphate-buffered saline (DPBS) and incubated in DAPI for 5 min followed by washing with DPBS. For SEM observation, the cells were washed with DPBS and fixed with 2.5% glutaraldehyde in PBS for 15 min at room temperature. After

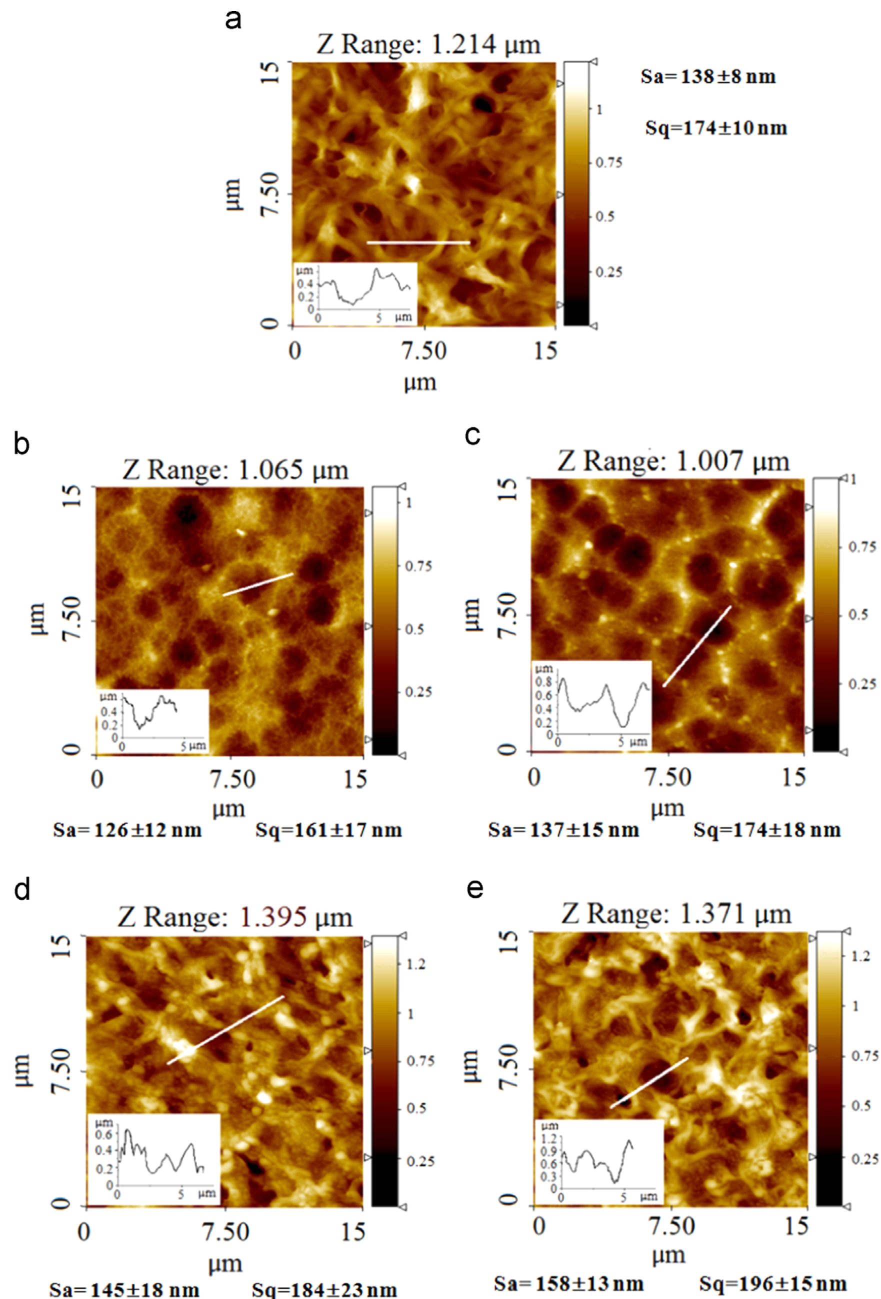

Fig. 1. Typical AFM images of the untreated (a) and oxygen plasma-treated P3HB at 100 W (b) and 150 W (c) and the ammonia plasma-treated samples at 100 W (d) and 150 (e).

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