Low-temperature argon and ammonia plasma treatment of poly-3-hydroxybutyrate films: surface topography and chemistry changes affect fibroblast cells *in vitro*

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Abstract

Poly-3-hydroxybutyrate (PHB) films were plasma-treated using pure NH_3, pure Ar or mixtures of the two different proportions (20%, 30%, 40%, 50%, 70% NH_3 in Ar). Surface chemistry and surface topography changes of PHB films were observed after plasma processing in all plasma regimes. The XPS results confirmed the absence of chemical modification in the case of pure Ar plasma treatment. Nitrogen-containing groups (e.g., N–C=O) were detected on the surfaces of P3HB films treated with NH_3-containing plasma. The surfaces of the untreated P3HB films were hydrophobic, and plasma treatment turned the surfaces hydrophilic, irrespective of the treatment. A significant decrease in the contact angle and an increase in the free surface energy were observed. An insignificant surface ageing effect was observed when P3HB samples were exposed to air for 10 days. In NIH 3T3 mice fibroblast cells, cell adhesion was significantly improved after plasma treatment in an Ar atmosphere, which is likely related to the fact that there was a surface ξ potential of 88.6 mV at neutral pH, causing a cleavage of the polymer chains and an increase in surface roughness.

Keywords: plasma treatment, dielectric barrier discharge, surface etching, wetting behaviour, cell adhesion

1. **Introduction**

Biodegradable polymers are of interest, particularly for the fabrication of biodegradable bone implants [1]. Of all the known biodegradable materials, polyesters of hydroxycarboxylic acids, i.e., polyhydroxyalkanoates (PHAs) are potentially the most important emerging class of materials for diverse biomedical applications. These polymers exhibit good biocompatibility and have useful physical and mechanical properties that are similar to hard tissues in the human

body, making them promising for use in reconstructing bone. Despite all the advantages of PHAs, they have not yet achieved wide clinical use due to the absence of osteoconductive properties on their surfaces.

Poly-3-hydroxybutyrate (P3HB), a homopolyester of 3-hydroxybutyric acid, is the most well studied PHA. P3HB is slowly biodegraded in an organism, producing non-toxic 3-hydroxybutyric acid; therefore, it can be used as a material for a wide spectrum of biomedical applications from sutures and drug delivery systems to matrices for tissue engineering and reconstructive surgery [2]. However, the high crystallinity of P3HB (over 70 %) leads to high rigidity and brittleness in products, and products show low resistance to stretching [3]. Moreover, the bioresorption of this polymer takes from several months to a few years, which can be undesirable when the healing of damaged tissue occurs quickly and triggers a foreign body reaction [4]. As a representative of the PHA family, P3HB has hydrophobic surface characteristics that can prevent normal interaction of the polymer surface with biological media in the organism, and this can disturb cell adhesion [5]. The wettability of P3HB can vary with the structure and composition of the surface. Wettability can vary with factors such as surface topography, the content of the crystalline and amorphous phases, and the presence of polar functional groups with a specific charge [6].

Radio-frequency (RF) plasma is usually used to treat the surfaces of different polymers [7, 8]. Comparisons between different techniques for polymer surface treatment reveal that plasma treatment is particularly versatile because modification is limited to the upper surface layers of the material, leaving the bulk properties unaltered [8, 9]. Reactive gases make it possible to obtain the required functional groups on the P3HB surface and to impart other properties such as bioactivity [10]. There are only limited data on the influence of different working parameters of plasma, such as the type of gas, power, and pressure on the changes in the surface properties of PHA. Data on wettability are particularly limited [8].

The effect of plasma treatment and surface ageing of polymers on cell behaviour has been

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investigated elsewhere [11-13]. However, detailed analysis of the surface chemistry changes and mechanisms has not been conducted. In particular, it is not well understood how we can choose a more appropriate plasma-treatment atmosphere to achieve better cell performance. Thus, the objective of this study is to modify P3HB thin films with a low-temperature RF plasma using different inert and reactive gas atmospheres (oxygen, ammonia and mixtures thereof) to improve surface wetting behaviour with hydrophilic polar functional groups.

2. Materials and Methods

2.1. P3HB film preparation

P3HB (average molecular weight 720 kDa, polydispersity index 3.60) films were obtained by a microbial biosynthesis with the use of *Cupriavidus eutrophus* B-10646 stamm, which is included in an all-Russian collection of commercial microorganisms [14]. Polymer films in the form of discs with diameter 10 cm and thickness 60-70 μm were obtained using homopolymer P3HB by the evaporation method from a solution in chloroform. First, 40 mL of 2 % polymer solution was heated to 35 °C and poured into clean Petri dishes. Then, the films were allowed to dry for 3-5 days under normal conditions in a laminar flow hood.

2.2. Plasma Processing

The P3HB films were treated in a custom-made dielectric barrier discharge (DBD) plasma system equipped with an RF power generator (13.56 MHz). Various working gases were used such as Ar (99.99%) and NH_3 (99.99%). Plasma treatment was performed in Ar − 100 % with a gas flow rate of 30 standard cm^3 min^{-1}, NH_3 - 100% (30 standard $cm^3 \cdot min^{-1}$), and Ar was used with 70, 50, 40, 30, and 20 % NH_3 at gas flow rates of 21, 15, 12, 9, and 6 standard $cm^3 \cdot min^{-1}$. The P3HB films were treated under a pressure of 30 Pa and at RF power of 30 W for Ar plasma and at 50 W for NH_3 and Ar/NH_3 plasma. The treatment time was always 5 min.

2.3. P3HB film characterization

2.3.1. Atomic Force Microscopy (AFM)

The topographies of untreated and modified P3HB films were analysed by atomic force microscopy (AFM, SOLVER) (NT-MDT, Russia) using a non-contact mode. In this study, 35-, 15- and 5-μm^2 images were recorded for each sample in three different locations. The roughness parameters (S_a - average roughness, S_q - root mean square, Sdr - surface area ratio between the interfacial area and projected area) were calculated using OPUS software (for 35-μm^2 images). In addition, the roughness factor was calculated by using the following equation: r = 1 + Sdr / 100. The Wenzel equation, cosθm=r cosθy (θm - measured contact angle, θy - Young contact angle), was used to derive the values of the Young contact angles using the measured values of the contact angles. We note that the Wenzel equation is based on the assumption that the liquid penetrates into the roughness grooves [15, 16].

2.3.2. Chemical analysis

2.3.2.1. X-ray Photoelectron Spectroscopy (XPS)

Prior to the measurements, the samples were exposed to ambient conditions. The surface chemical compositions of the untreated and plasma-treated PHB films were studied using X-ray photoelectron spectroscopy (XPS). XPS was performed using an unmonochromated XR-50 Mg K_α X-ray source (hv=1253.6 eV) from SPECS (Germany) at a base pressure of 1-2$\times 10^{-10}$ Torr. The analyser was set to a pass energy E_p = 50 eV and energy step E_s = 0.5 eV for the XPS survey spectra of the coatings, and it was set to E_p = 20 eV and E_s = 0.1 eV for higher-resolution spectra. The obtained spectra were analysed using CASA-XPS software [17]. The binding energy (BE) scale was calibrated by measuring the reference peak of C 1s (BE = 285 eV) corresponding to adventitious carbon contamination.

2.3.2.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were obtained using a Bruker (Germany) EQUINOX 55 infrared analyser equipped with an attenuated total reflectance (ATR) accessory providing analysis of the surface. The spectra of the P3HB films were recorded in the frequency range of 600 to 4000 cm^{-1}.

2.3.3. Wettability study

Static contact angles of the droplets of Milli-Q water were measured on untreated and plasma treated P3HB films using an optical contact angle apparatus (OCA 15 Plus Data Physics Instruments GmbH, Germany) and appropriate SCA20 software (Data Physics Instruments). All measurements were performed using the sessile drop method at room temperature 20 ± 1 °C. Average values in each case were calculated for a minimum of 10 water droplets (1 μL, 2 μL·s^{-1}). The calculations of surface free energy were carried out using the Owens–Wendt–Rabel–Kaelble (OWRK) method for three different media (Milli-Q water, diiodomethane and ethylene glycol).

2.3.4. Zeta potential analysis

The zeta potential was analysed by measuring the streaming potential for P3HB films at pH values from 2.5 to 10 using the SURPASS (Anton Paar) system and an electrolyte of KCl solution with concentration 0.001 M. The zeta potential was determined from the streaming potential at various flow rates.

2.3.5. Cell culture assays

For cell culture experiments, polymer discs were cut with a diameter of 15 mm. The samples were sterilized in 70 % ethanol alcohol for 30 minutes, then washed in modified Eagle's medium (DMEM) and placed in 24-cell plates for tissue culture (Techno Plastic Products AG). NIH 3T3 fibroblasts were seeded on polymer films with a density of 10^5 cells·cm^2. Cell adhesion, proliferation and growth were estimated. The experiments were performed in DMEM solution with the addition of 10 % bovine serum and antibiotics (streptomycin 100 μg/ml, penicillin 100

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ME/ml) (Sigma) in a 5 % CO_2 atmosphere at 37 °C for 7 days. The morphologies of fibroblasts were investigated using adhered cell staining with the help of the fluorescent DNA-probe DAPI (Sigma-Aldrich). After fixation, the cells were washed three times in Dulbecco's Phosphate-Buffered Saline (DPBS) and incubated in DAPI solution for 5 min, followed by washing in DPBS. The biocompatibility of P3HB films was investigated by an MTT-assay based on the ability of live cells to restore unstained forms of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT - reagent) to blue formazan, which is soluble in dimethyl sulphoxide. The optical density was determined using an iMark spectrophotometer (Bio-Rad, USA) after dissolving the MTT-formazan crystals in DMSO. All biological observations were conducted on 4 surfaces for each type of sample.

3. Results and discussion

3.1. AFM analysis

The morphology of biomaterials is one of the major factors controlling cell behaviour. It is known that the surface of an implant should reveal topographical features of up to 100 μm (cell size approximately 10-50 μm [18]) to promote the mechanical anchoring of cells and to provide new tissue growth [19,20]. Additionally, the nano- and sub-micro-roughness (up to 1 μm) of a material promotes the adhesion and proliferation of cells [18, 21]. Plasma treatment makes it possible to vary the nano- and micro-topographical dimensions of the surface via etching or polymer chain cleavage.

AFM analysis was performed on untreated and plasma-modified P3HB films to visualize their morphologies and reveal the roughness changes that occurred after plasma surface modification. The results are presented in Figures 1 and 2 and in Table 1. The AFM images show that topographical changes appear after 5 min of plasma treatment in different gas atmospheres, and

an increase in the roughness parameters is observed (Table 1). This includes the surface area ratio between the interfacial area and the projected area (Sdr), which had an effect on the increase of the roughness factor (r). These observed changes could be explained by taking into account different processes that occur on the P3HB surface. The surfaces of P3HB films before and after plasma treatment reveal microscale topographical features, which were similar in the cases of Ar, NH$_3$ and Ar/NH$_3$ plasmas (see Figs. 1 and 2).

3.2. Surface chemistry analysis

Figure 3 represents XPS survey spectra of P3HB films before and after plasma treatment in Ar, NH$_3$, Ar/NH$_3$ atmospheres. All the expected peaks (C 1s, O 1s and N 1s chemical states) were observed except for Ar 2p chemical state. However, this peak was observed in all high-resolution XPS spectra corresponding to P3HB films treated in Ar-containing plasma.

Table 2 represents relative atomic concentrations (%) of the elements, which were calculated using high-resolution XPS spectra for untreated and treated P3HB films. An increase in the C/O ratio was observed with increasing NH$_3$ content in the plasma atmosphere, as is also shown in Figure 4 In the case of P3HB films treated in Ar plasma, the decrease in the C/O ratio was observed in the treated but not the untreated P3HB film.

The calculated C/O ratio for the untreated PHB film was 2.2, which was greater than the theoretical value (2.0) [22]. However, in the case of poly(hydroxybutyrate-co-hydroxyvalerate) with an HV content of 8.8%, the authors of [23] observed that the C/O ratio was 2.65, which was significantly higher than the theoretical value of 2.04.

Plasma treatment of polymers usually causes the atomic concentration of oxygen to increase (in polymers without oxygen in their structure) [24]. P3HB has oxygen in its chains; therefore, plasma treatment leads to a decrease in oxygen content in the surface layer. The C/O ratio should thus increase due to cleavage of the polymer chains and subsequent surface ablation [25]. Conventional thermal degradation of P3HB can lead to the formation of such volatile products

with increased carbon content such as propene and propenyl-2-butenoate [26]. A loss of carbon groups can be expected because they are more numerous than oxygen-containing groups, and there will be a subsequent decrease in volatile carbon-containing components. This was observed in the case of Ar-plasma treatment. However, plasma treatment leads to the formation of new active centres (e.g., radicals), which can react with air and form new chemical groups containing both oxygen and nitrogen [25]. It is likely that chemical groups containing nitrogen are formed on the surface of the P3HB films treated in an NH_3 plasma atmosphere, which can explain the increase in the C/O ratio as the concentration of NH_3 in the Ar atmosphere increases during plasma treatment. It has been suggested that the interaction of ionized Ar with the P3HB polymer chains causes cleavage to occur rather than surface chemistry changes. During the ammonia plasma interaction with the oxygen groups of PHB at the sites of chain cleavage, a partial exchange of terminal oxygen groups for N-groups (e.g., OH for NH_2) is likely to occur, which results in a decrease in oxygen concentration in the surface layers and an increase in the C/O ratio (Figure 4). The XPS results confirmed the presence of nitrogen on the surfaces of the treated P3HB films in NH_3-containing plasma.

The high-resolution XPS spectra of the investigated samples are presented in figure 5. The C 1s spectrum of untreated PHB film (Figure 5a) was decomposed into tree component peaks showing the presence of different oxidation states of carbon: 285 eV, 286.7 eV and 288.8 eV, due to C–C(H), C–O and O=C–O components, respectively [22,23]. After Ar plasma treatment (Figure. 5b), a high-resolution XPS C1s spectrum shows the same peaks as for the untreated P3HB film. However, a comparison of figures 5a and b reveals the increase of the C–O component. This increase could lead to a decrease in the C/O ratio after pure Ar plasma treatment in comparison with the untreated P3HB film. After ammonia plasma treatment (Figure 5c), the XPS scan of the carbon region shows a spectrum with one additional peak, which is observed at 287.6 eV and corresponds to the typical binding energy for an amide group (N–

C=O) [22,23]. When Ar and NH$_3$ gases were used together in plasma with different concentrations (Figure 5 d-f), an XPS scan of the C 1s region showed similar spectra as for pure ammonia plasma treatment, i.e., C–C(H), C–O, O=C–O and N–C=O components.

Figure 6 shows the high-resolution XPS spectra of the N 1s chemical state that was observed after NH$_3$ plasma treatment. Despite the decrease in NH$_3$ content in the plasma atmosphere, a difference in the N 1s chemical state was not observed. All N 1s peaks were similar. The results summarized in Table 2 show insignificant differences in N 1s content between samples. There were no peaks in the N 1s region after Ar plasma treatment or for untreated P3HB samples. According to the literature, the observed peak of nitrogen at 399.9 eV corresponds to amide groups (N–C=O) [22,23]. Thus, the high-resolution spectra of the N 1s peak confirm the addition of different nitrogen-containing molecules to the surface during NH$_3$ plasma treatment. It has been reported that an NH$_2$ radical can recombine with radicals on carbonyl groups to form amides during the NH$_3$ plasma treatment of P3HB [22].

3.3. Contact angle measurements

The surface wettability of the modified polymers indicates particular changes in the chemical and physical nature of the surfaces, which were usually altered by plasma treatment. Due to wetting associated with the surface energy and surface tension, any manipulations of the chemical compounds on the surface of P3HB lead to changes in contact angles. For biological applications, wettability is an important factor for cell adhesion and proliferation because various cell types behave differently on different surfaces. It has also been reported that an optimal water contact angle for cell adhesion is in the range of 50-70° [27]. Plasma treatment of hydrophobic polymers allows us to prepare a hydrophilic surface [9]; thus, plasmas have been used to improve the biocompatibility of polymers [28,29] and to control surface chemical composition [28].

The contact angles of the modified P3HB surfaces were measured immediately after plasma

treatment and after samples were preserved in air for 10 days. Figure 7 shows the contact angle changes of the P3HB after Ar and NH$_3$ plasma treatment. Untreated P3HB had a contact angle of 99°±1.0° and thus a hydrophobic surface. After NH$_3$ plasma treatment, the contact angle decreased significantly to 49.5º±1.2º, which produced a hydrophilic surface. After pure Ar plasma treatment, the contact angle was also significantly reduced relative to the untreated P3HB film; however, it remained greater (65.2º±3.5º) than that after pure NH$_3$ plasma treatment. The decrease in contact angle can be explained by the increase in the number of hydrophilic C-O groups on the surface (Figure 5b), as observed in the XPS spectra in the case of Ar plasma. The hydrophilic surface of the NH$_3$ plasma-treated polymers can be explained by taking into account the formation of new functional N-C=O groups (Figure 5c).

The formation of new functional groups was also confirmed by measuring the surface free energy. For untreated P3HB films, the dispersion component of the surface energy was comparable to the total surface energy, and the polar component was negligible (Figure 7b). For untreated P3HB surfaces, the polar component was 0.44 mN/m, and for the surfaces treated in pure Ar plasma, the polar component was 12.26 mN/m. The polar component was 20.32 mN/m for NH$_3$ plasma. There were significant increases in the polar component after all plasma treatments, and no significant changes occurred in the dispersion component.

The contact angles of the modified P3HB films after storage in air showed no significant decrease in surface wettability and energy. In contrast, the reduction of the contact angles was similar to what was observed on the first day due to the increase of the polar component of the surface energy.

Wettability is affected by a combination of topographical changes and surface chemistry changes. Based on the results presented in Figure 7, it can be concluded that both surface chemistry and topography changes affect the contact angels in the case of NH$_3$ plasma treatment. Because no chemistry changes were observed after Ar plasma treatment, the decrease in the

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contact angle was likely connected to the surface topography changes and polymer cleavage. However, taking into account the statistically significant difference between the results obtained for wettability and roughness in pure Ar and pure NH_3, we conclude that surface chemistry changes played a more significant role in the decrease in the WCA values (Figure 7a) and the increase in the surface free energy (Figure 7b).

Because Ar is an inert gas, the surface etching is mainly caused by Ar ion bombardment. In the case of reactive NH_3 gas, chemical reactions can also occur, which result in the removal of newly formed molecules from the polymer surface. Thus, in a mixture of Ar and NH_3 gases, the P3HB surface is etched due to reactive ion bombardment.

In Figure 7, the water contact angle, Young's contact angle and surface free energy of the samples treated in Ar/NH3 plasma are shown. The water contact angle was reduced relative to the untreated P3HB; it was 48º at 20 % NH_3 in Ar. It was 43º at 30 – 50 % NH_3, and it was 56º at 70 % NH_3. The surface energy of the modified P3HB increased significantly for all mixtures of NH_3 in Ar plasma. At first, the modified surfaces showed an increase in the polar component up to 22-25 mN/m (for 20-50 % NH_3), and some showed a decrease in the dispersive component. After 10 days of exposure to air, the dispersive component showed some recovery, and there was a moderate decrease in the polar component of the surface energy.

Considering the calculated values of the Young contact angles, we conclude that both the surface chemistry and surface roughness changes following plasma treatment played significant roles in determining the surface wettability of the polymers. The effect of the surface roughness (Table 1) on wettability was clearly demonstrated in the case of Ar plasma treatment because, in this case, only surface etching was observed, without any surface chemistry changes.

3.4. Zeta potential measurements

An analysis of the zeta potential (ξ) of the modified films in different gas atmospheres revealed

evidence of chemical modification of the P3HB surfaces. The zeta potential and pH measurements for untreated and treated P3HB in Ar and NH_3 plasmas are presented in Figure 8. The isoelectric points (IEP) calculated for ξ potentials at pH=7 are presented in Table 3. After plasma treatment, the ξ curves shift to lower pH values: 3 and 5 for the Ar and NH_3 plasmas, respectively. The zeta potential shifts revealed ξ potentials of -46.5 mV for untreated P3HB, -88.6 mV after Ar modification and -69.8 mV after NH_3 modification at a physiological pH of 7. This tendency towards lower ξ values is typical for acidic molecular groups and can be attributed, in particular, to COO radicals [30]. The ξ measurements provided evidence not only of the surface potential for biomolecular interaction but also the electrolyte pressure on the surface (600 mbar), which is a reliable indicator of film stability and durability. Neither untreated nor modified P3HB films showed signs of film degradation due to zeta potential measurements.

3.5. Cell viability

Surface plasma treatment of the polymer films resulted in changes in NIH 3T3 mouse fibroblast adhesion behaviour. For example, there were changes in the number of adhered cells and cell viability. Fluorescent microscopy of the surface fibroblasts revealed a sufficiently high level of adhered viable cells in all samples. Even cells looked similarly, cell density was significantly higher on the treated surfaces when the surfaces were observed visually (Figure 9a).

A similar effect was quantitatively observed during the MTT test. The effect of plasma treatment was seen on day 1 after cell seeding. On day 7, the number of cells on the plasma treated samples was higher than in the control (Figure 9b). The maximal number of cells was observed in the case of polymer films treated in a 100 % Ar atmosphere. The results for cell adhesion in the case of 100 % NH_3 and 50%/50% Ar/NH_3 were similar. In general, the results correlate well with the results obtained earlier for plasma treatment of polymer samples in ammonia under different conditions [8].

Ammonia plasma treatment can be expected to produce a hydrophilic surface due to chemical grafting of ammonia groups, which stimulates the adhesion of fibroblasts. Also, the maximal roughness increase was registered for Ar/NH_3 plasma treated samples when NH_3 concentration was 20-30% (Table 1). However, the results showed statistically significantly better adhesion of cells when Ar plasma was used relative to untreated samples, which is in good agreement with the values of the ξ potential. These values were more negative for the Ar plasma-treated surfaces.

The hydrophilicity of the surface plays an important role in protein adsorption, but its role in determining cell behaviour is still under discussion. A previous study [31] reported that the best cell adhesion is typically obtained with hydrophobic materials with $\tau\circ$ < 30 dyn/cm. Other studies showed that a hydrophilic surface is required for hydrophobic materials to provide attachment and cell growth. In general, extreme levels of hydrophobicity and hydrophilicity are thought to be disadvantageous for cell adhesion, whereas moderate values promote effective protein adhesion [32]. At the same time, the presence of proteins at the surface provides successful cell adhesion [33,34]. Just a change of surface chemical composition can be one of key factors for protein adhesion (and, therefore, for cell growth) even roughness remains unchanged [35]. Many of the well-investigated polymers 31,36-38] have water contact angles at which the best adhesion of different mammalian cells can be observed. These angles were in the range of 30° to 50°, which is lower than the values (70-80°) observed after Ar plasma processing and similar to the range of the samples treated in ammonia plasma. These results can be explained not only by taking into account the hydrophilic nature of the plasma-treated surfaces but also by considering their electrokinetic characteristics. The largest values of the ξ potential (Figure 8) were observed after Ar plasma treatment, and treatment in ammonia plasma resulted in ξ potential values that were moderate compared with the intact polymer surface.

Conclusions

P3HB films were treated in different plasma atmospheres such as pure NH_3, pure Ar and mixtures thereof. Surface chemistry and topography changes were observed, such as increases in the roughness parameters Sa and Sq caused by plasma surface etching of the P3HB polymer films in the case of NH_3 plasma, and polymer chain cleavage was observed in the case of pure Ar atmosphere. No surface modifications were observed aside from polymer chain cleavage in the case of Ar plasma treatment. However, chemical groups containing nitrogen (e.g., N–C=O) were formed on the surfaces of P3HB films treated in an NH_3 atmosphere, which could explain the increase in the C/O ratio with the increase in NH_3 concentration in the Ar atmosphere. Insignificant changes in both surface wettability and energy were observed after all plasma-treated samples were exposed to air for 10 days. However, significant decreases in the contact angles and increases in surface energy were observed compared with cases of untreated P3HB. Increased NIH 3T3 fibroblast cell adhesion was observed in the case of Ar plasma treatment, and this may be attributed to the ξ potential of 88.6 mV at neutral pH, which is lower than what is observed with other treatment procedures and increases the surface roughness compared with untreated P3HB samples. The cleavage of the polymer chains and the surface roughness also increase. Thus, the results obtained show that plasma-treated P3HB films could be used in different clinical applications, and they show that it is possible to adjust the surface chemistry and wetting behaviour of the films.

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Figures and tables captions:

Figure 1. AFM image of untreated P3HB (a) and modified P3HB films in Ar (b) and NH_3 (c) plasma.

Figure 2. AFM image of modified P3HB films in a plasma mixture of Ar/NH_3: (a) Modified P3HB in a plasma mixture of Ar/NH_3 (80/20), (b) Modified P3HB in a plasma mixture of Ar/NH_3 (70/30), (c) Modified P3HB in a plasma mixture of Ar/NH_3 (60/40); (d) Modified P3HB in a plasma mixture of Ar/NH_3 (50/50); (e) Modified P3HB in a plasma mixture of Ar/NH_3 (30/70).

Figure 3. XPS survey spectra of untreated and Ar, NH_3, and Ar/NH_3 plasma-treated P3HB films.

Figure 4. The dependence of C/O ratio on the concentration of NH_3 in plasma in comparison with the theoretical value, measured value from untreated and treated P3HB film in Ar plasma.

Figure 5. Typical high-resolution XPS spectra of the untreated P3HB (a) and treated films obtained using different plasmas: (b) pure Ar, (c) 100% NH_3, (d) 70-30 % Ar/NH_3, (e) 60-40 % Ar/NH_3, (f) 50-50 % Ar/NH_3.

Figure 6. Typical high-resolution XPS spectra of P3HB films treated with different plasmas: (a) pure NH_3, (b) 70-30 % Ar/NH_3, (c) 60-40 % Ar/NH_3, (d) 50-50 % Ar/NH_3

Figure 7. The water contact angle, Young's contact angle (a) and surface free energy (dispersive component –filled in color and polar component - dashed patterns) (b) depending on Ar and NH_3 plasma treatment. Storage times in air after plasma treatment were 1 day and 10 days.

Figure 8. Zeta potential vs pH curves corresponding to untreated and modified P3HB films.

Figure 9. Analysis of growth of NIH 3T3 fibroblasts on P3HB polymer films treated in low-temperature plasma. (a) MTT quantification on days 1, 3 and 7. Data are shown as the mean ± confidence interval ($P \leq 0.1$); * and ** denote significant differences with *$P \leq 0.1$ and **$P \leq 0.05$, n = 3. (b) Fluorescent microscopy images of adherent fibroblasts (FITC staining).

Table 1. Effect of plasma treatment on P3HB roughness parameters.

Table 2. Relative atomic concentrations (%) of the chemical states observed in samples.

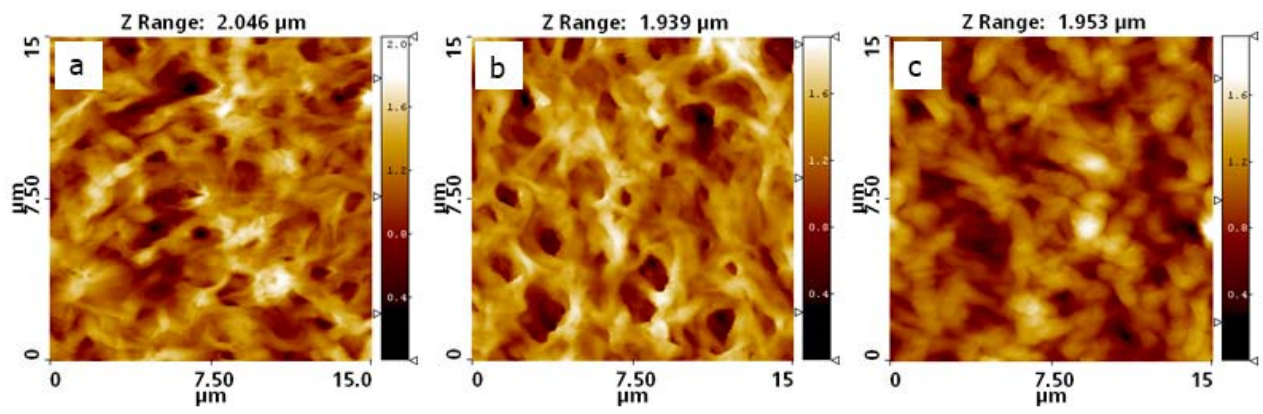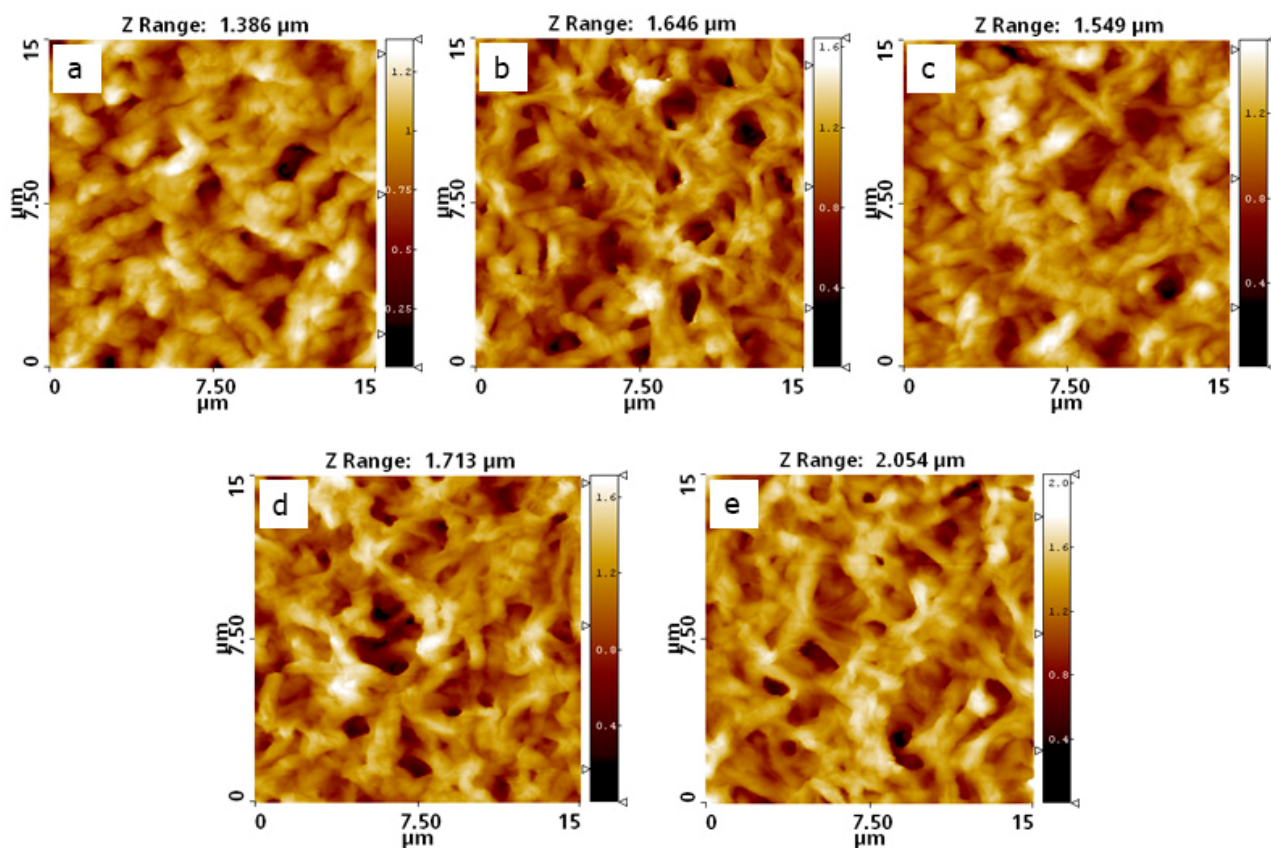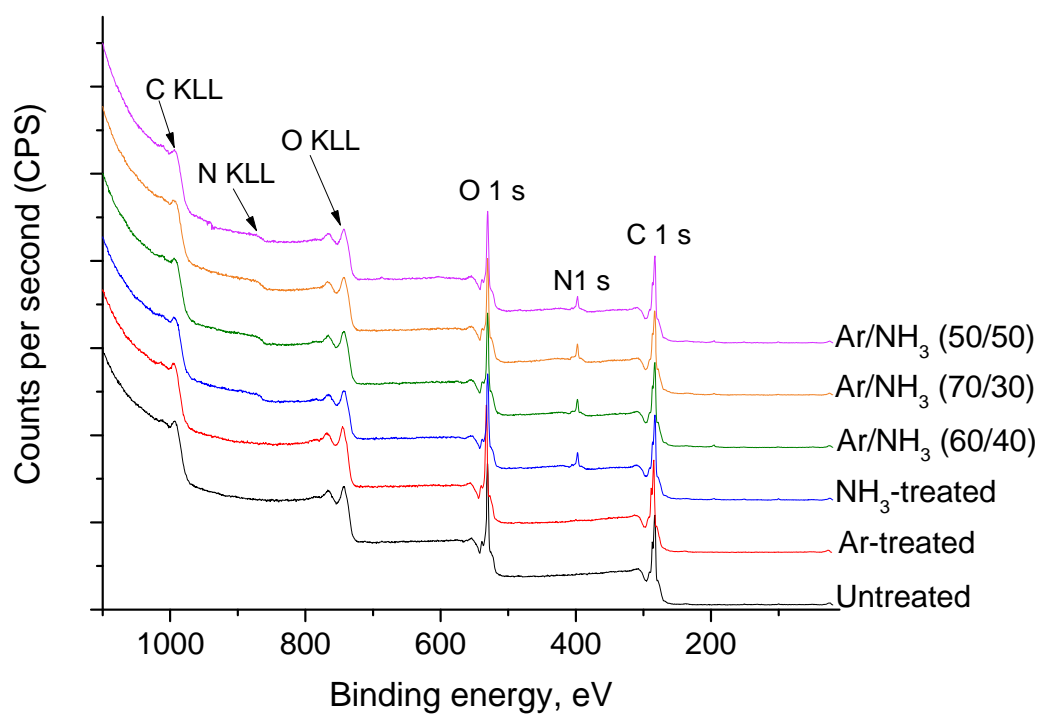Table. 3. The ξ potential and IEP values for different plasma atmospheres.
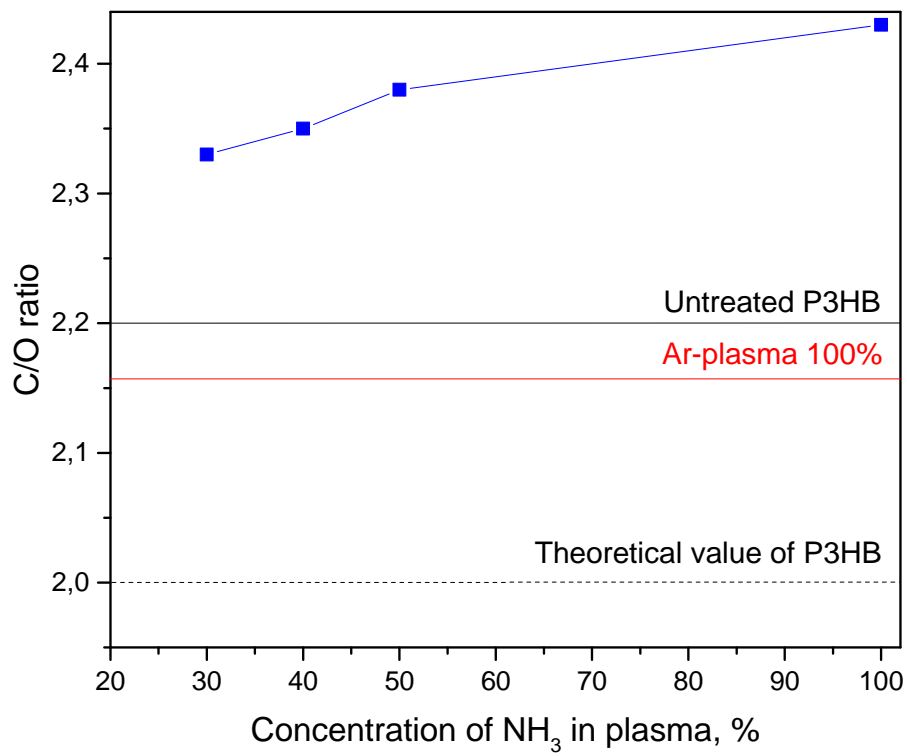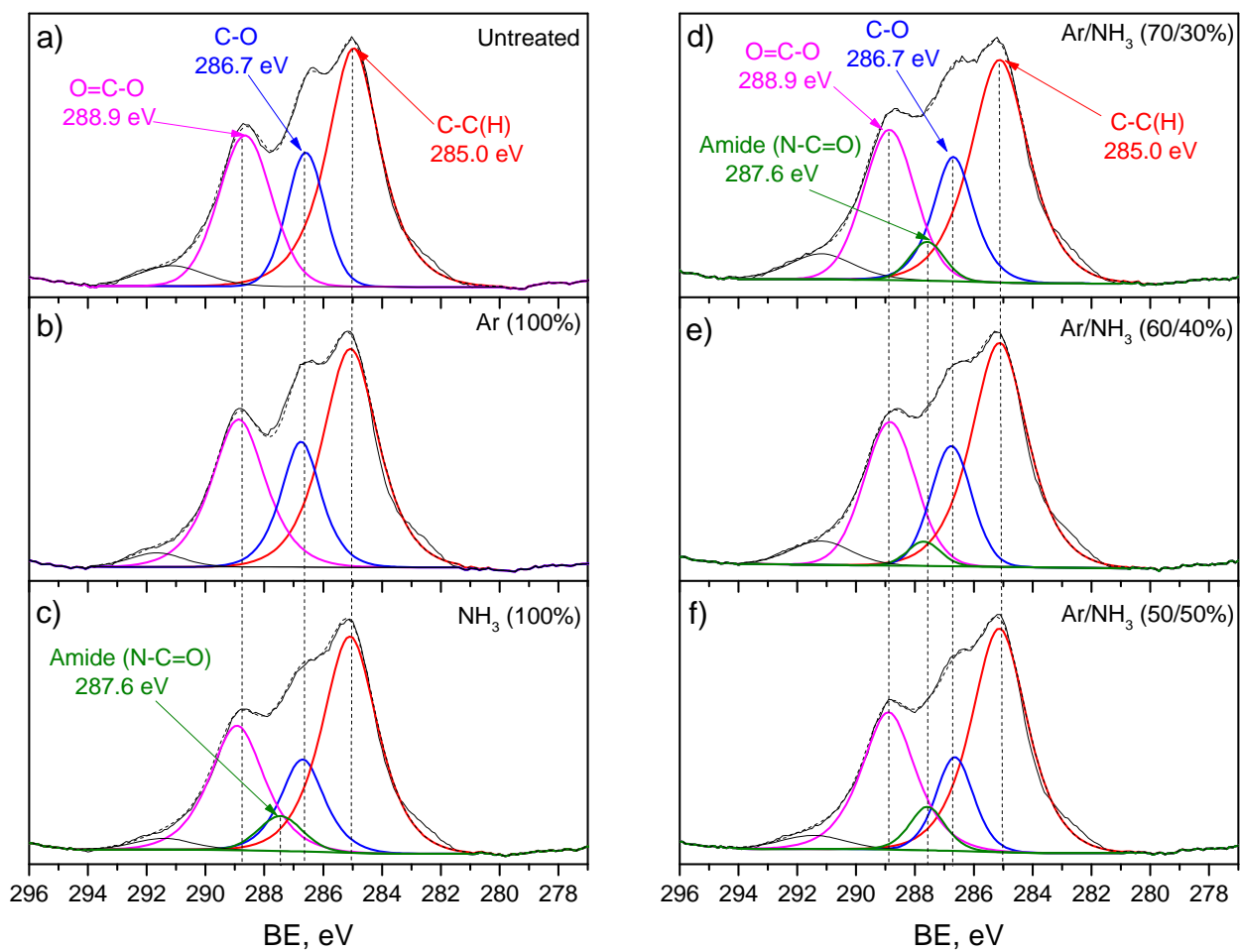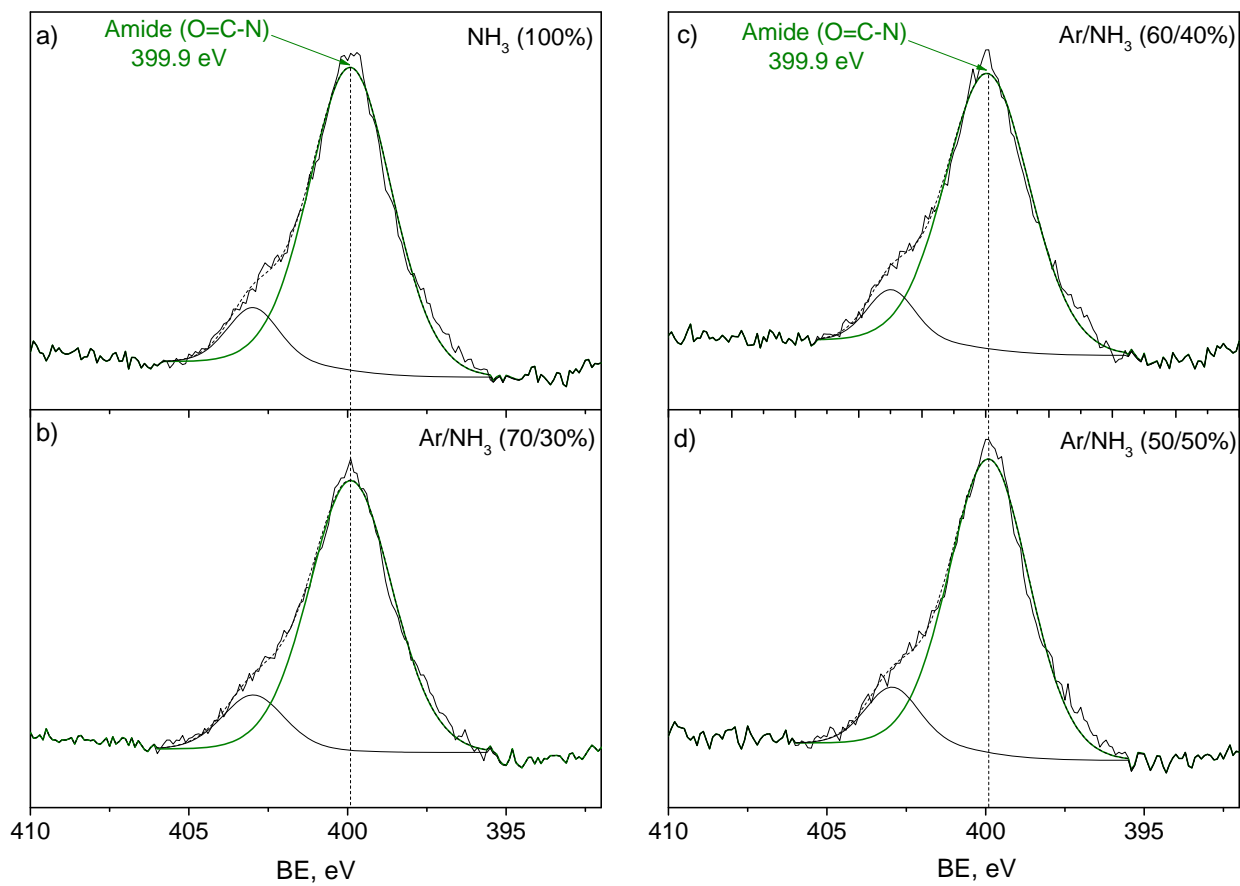
Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.
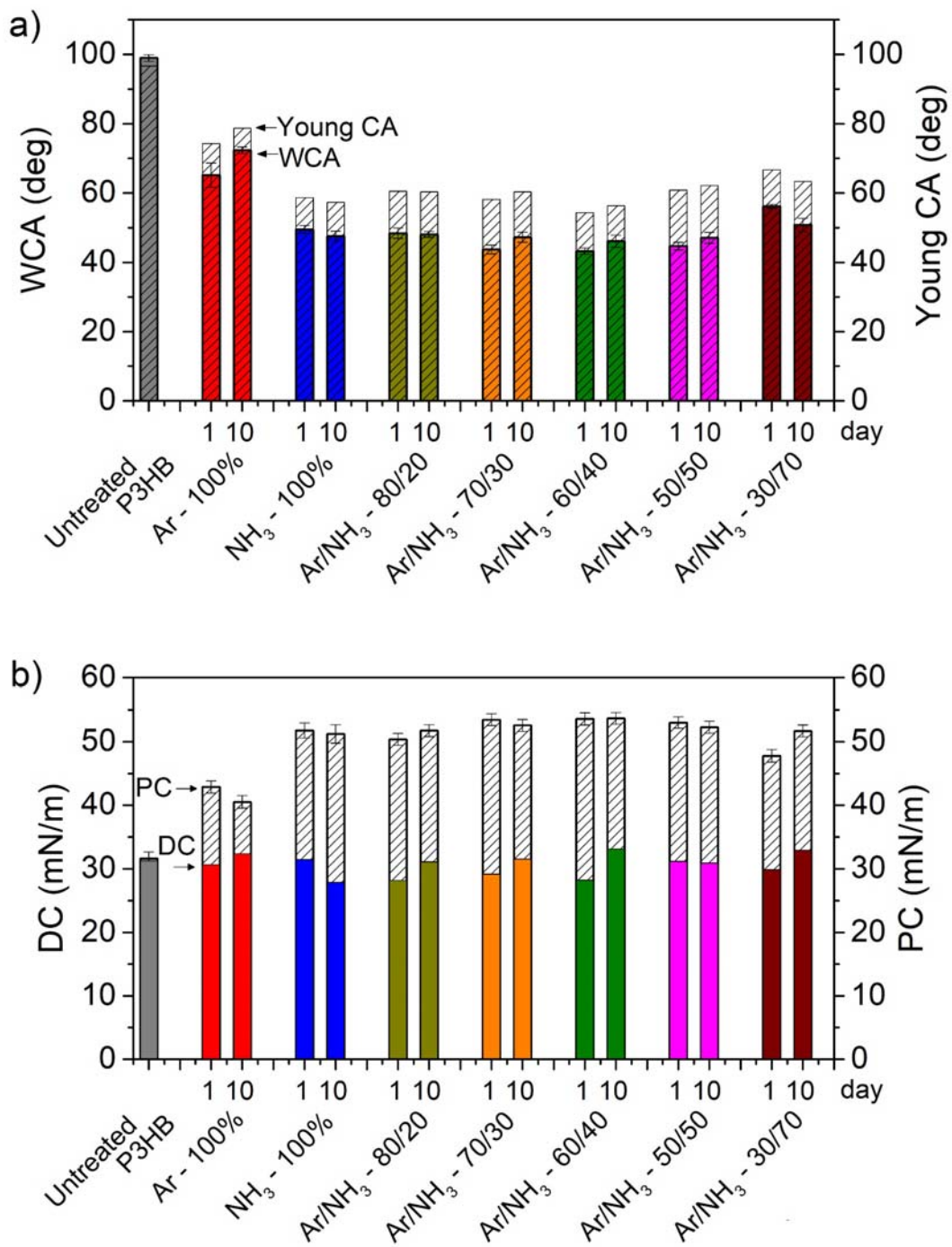
Figure 7.
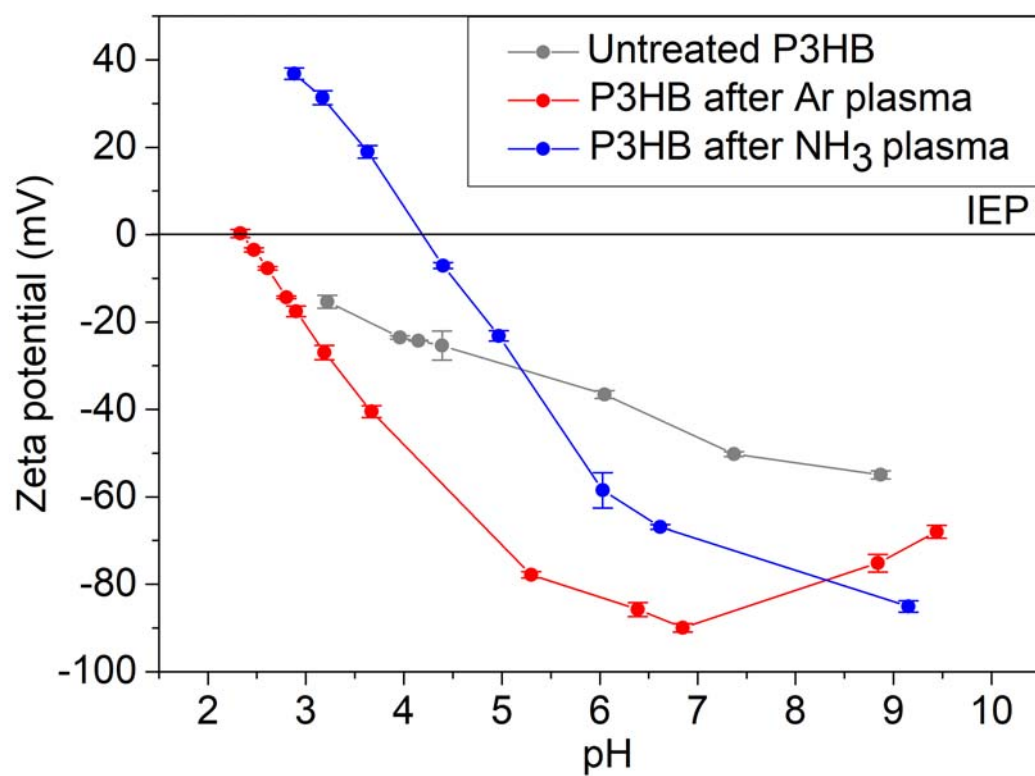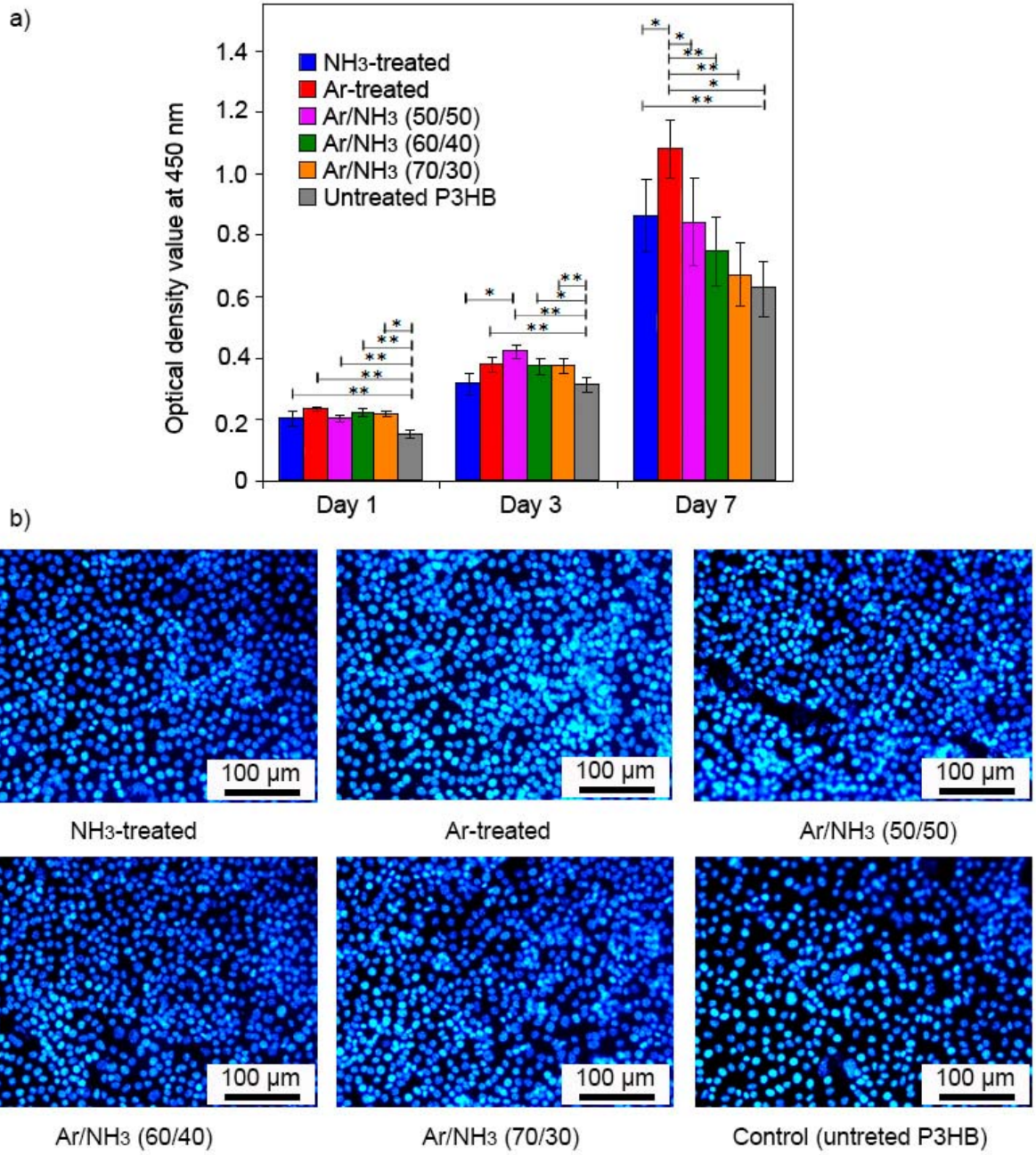
Figure 8.

Figure 9.

Table 1.

Type of surface processing	Sa, nm	Sq, nm	Sz, nm	Sdr, %	r
Untreated P3HB	247±31	310±42	2187±245	34.9	1.35
P3HB, Ar plasma	260±28	325±30	2131±442	55.4	1.55
P3HB, NH$_3$ plasma	260±13	323±19	2065±354	24.8	1.25
P3HB, Ar/NH$_3$ (80/20) plasma	367±154	448±177	2642±699	34.7	1.35
P3HB, Ar/NH$_3$ (70/30) plasma	334±95	412±111	2393±762	36.7	1.37
P3HB, Ar/NH$_3$ (60/40) plasma	281±80	349±95	2223±374	25.1	1.25
P3HB, Ar/NH$_3$ (50/50) plasma	242±37	304±44	2106±631	51.7	1.52
P3HB, Ar/NH$_3$ (30/70) plasma	296±17	369±21	2483±194	40.9	1.41

Table 2.

№	Substrates	Relative atomic concentrations, %				C/O ratio
		C 1s	O 1s	Ar 2p	N 1s	
1	P3HB	68.75	31.25	0.00	0.00	2.2
2	Ar-treated P3HB	67.58	31.3	1.12	0.00	2.16
3	NH$_3$-treated P3HB	65.93	27.12	0.00	6.96	2.43
4	Ar/NH$_3$ (60/40)-treated P3HB	65.24	27.82	0.63	6.31	2.35
7	Ar/NH$_3$ (70/30)-treated P3HB	64.52	27.71	0.88	6.89	2.33
8	Ar/NH$_3$ (50/50)-treated P3HB	65.58	27.6	0.88	5.95	2.38

Table 3.

	ξ potential at pH=7, mV	IEP
P3HB	-46.5	-
Ar plasma	-88.6	2.34
Ar/NH$_3$ (80/20%) plasma	-53.6	4.91
Ar/NH$_3$ (70/30%) plasma	-61.7	4.52
Ar/NH$_3$ (60/40%) plasma	-62.3	4.66
Ar/NH$_3$ (50/50%) plasma	-55.6	4.22
Ar/NH$_3$ (30/70%) plasma	-64.5	4.17
NH$_3$ plasma	-69.8	4.22