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Activation of polylactide films by cold plasma dielectric barrier discharge to improve the interaction of fibroblasts

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Abstract. This work focuses on DBD (dielectric barrier discharge) surface modification of polylactide (PLA) films. The film samples were treated in a cold plasma in order to optimize their biological properties and interaction with human skin fibroblasts. Optimal film processing modes for the increased proliferative activity of cells have been found.

Keywords: DBD plasma, polylactide, fibroblast, modification, contact angle

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Материалы конференции
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Активация полилактидных пленок диэлектрическим барьерным разрядом в холодной плазме для улучшения взаимодействия с фибробластами

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Аннотация. В данной работе была проведена модификация поверхности полилактидных пленок PLA с помощью барьерного разряда с целью оптимизации их биологических свойств при взаимодействии с культурой дермальных фибробластов человека. Найдены оптимальные режимы обработки пленок в газовом разряде, позволяющей повысить пролиферативную активность клеток.

Ключевые слова: барьерный разряд, плазма, полилактид, фибробласты, угол смачивания

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Introduction

Polylactide is a weakly hydrophobic polymer [1] (water contact angle (WCA) ranges from 55° to 80°), its surface is prone to nonspecific protein adsorption, which promotes bacterial adhesion [2]. Therefore, it is important to improve the wettability of the surface in order to improve the biocompatibility and antibacterial properties of PLA, which define the biological processes occurring in the surface layers [3].

Plasma treatment is a popular method of polymer surface modification [4]. Plasma is an ionized gas achieved in a high electric field, which can be high-temperature or low-temperature at different pressure [5]. The DBD (dielectric barrier discharge) modification occurs only on the surface of the material, the volume properties do not change [6]. Moreover, it is a process that changes the surface in a controlled, reproducible and homogenous way, so it can be used even in cases where the surface has an irregular geometry [7]. The main effects of plasma treatment are [5]:

- Surface cleaning, removal of organic contaminants
- Surface degradation (etching)
- Cross-linking
- Surface functional groups modification.

The occurring processes depend on the gas used for plasma production and determine physical and chemical properties of the sample surface [8]. For example, when chemically active gases such as oxygen, fluorine or ammonia are used, chemical changes occur; in case of oxygen, new peroxide, hydroperoxide, carboxyl or hydroxyl groups are formed on the surface, which leads to the immobilization of other compounds [9]. Inert gas plasmas, produced from helium or argon, enable the process of extensive cross-linking. In any case, DBD creates numerous reactive particles such as ions, radicals, electrons, photons and other excited particles, so other secondary reactions should be expected.

The goal of this work was to optimize the surface modification method of PLA films in a DBD (in air at atmospheric pressure). The aim of this modification was to improve the biocompatibility and bioactivity of materials during the interaction with human skin fibroblasts.

Materials and Methods

The objects of this study are samples of polylactide films, which thickness was 25±5 μm. The samples were produced using a DSM Xplore micro-extruder and a DSM Film Device Machine.

Dielectric barrier discharge (DBD) was generated in the ionization chamber consisting of ceramic plates with electrodes divided by air medium (the gap thickness h = 1 mm). Partial discharges that modified the polymer film surface appeared in the air gap. The cell capacitance was 8.8 pF; the electrode area was 11 cm^2. To provide sufficient amounts of oxygen, air was blown through the ionization chamber during plasma treatment of polymer films. Partial discharges in this cell developed uniformly over the whole surface of ceramic plates. The polylactide film was placed into the gas-discharge gap, as shown in Fig. 1. The source of high-voltage signals was a TVS-110 flyback transformer with Zero Voltage Switching (ZVS) driver in the primary coil. The schematic diagram of the DBD setup is presented in Fig. 1.

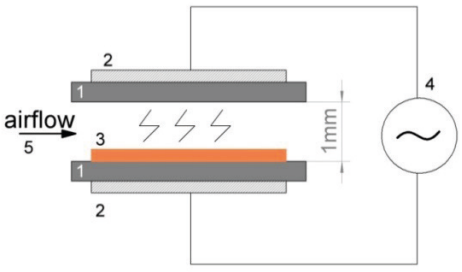

Fig. 1. Schematic of the DBD setup: ceramic plates *1*; electrodes *2*; PI film sample *3*; high-voltage generator *4*; fan *5*

The voltage providing stable activity of partial discharges was 2.6–2.7 kV at a frequency of 25 kHz. Use of high-frequency alternating voltage caused an increase in the frequency of partial discharges. The repetition frequency of discharges was 40 kHz. As such, the energy densities employed in this work were within the range 0.01–8.6 J/cm^2. The treatment time varied from 0.5 to 10 min.

Using a ZEISS Axio Scope.A1, the changes in the surface of PLA films after DBD modification were spotted.

The water contact angle (WCA) of a polylactide film was determined by the sessile drop method with the use of a DSA30 instrument (Kruss, Germany) in 24 h after plasma activation. The measurements were carried out at room temperature using 17 μL drops of distilled water and hexadecane. The WCA values were calculated in 5 s after precipitation of drops using the DSA4 software. The values were determined in 5 areas randomly distributed over the surface.

Mechanical properties were investigated using an Instron 5943 testing machine; the sample length was 20 mm, and stretching rate was 1 mm/min. 10 measurements were taken at each point. Each experiment was repeated twice.

To analyze the biocompatibility of materials *in vitro*, a strain of skin fibroblasts from a healthy donor (collection of cell cultures of the Institute of Cytology RAS) was used. The cells were cultured in a nutrient medium in a CO_2 incubator (Thermo Fisher Scientific, USA) at a temperature of 37 °C, a CO_2 concentration of 5% and high humidity.

To determine the viability and proliferative activity of cells after 4 days of cultivation on the surface of experimental samples, a MTT test was used. The nutrient medium was replaced with a solution of MTT (thiazolyl blue tetrazolium bromide) (Thermo Fisher Scientific, USA) and the samples were incubated for 2 hours.

After the incubation, the solution was removed and the formed formazan crystals were extracted into DMSO (dimethyl sulfoxide). The optical density of the solution was measured using a Spectrastar Nano spectrophotometer (BMG Labtech, Germany) at a wavelength of 570 nm. The number of viable cells was estimated by the optical density.

Results and Discussion

The effect of DBD exposure time on the microrelief of PLA films was recorded using a light microscope, micrographs are shown in Fig. 2.

Fig. 2 suggests that an increase in the DBD exposure time changes the relief of the films significantly. The density and depth of surface defects increase with the processing time. The size of the craters ranges from 1 microns to 5 microns. The developed film roughness will provide better adhesion of fibroblasts.

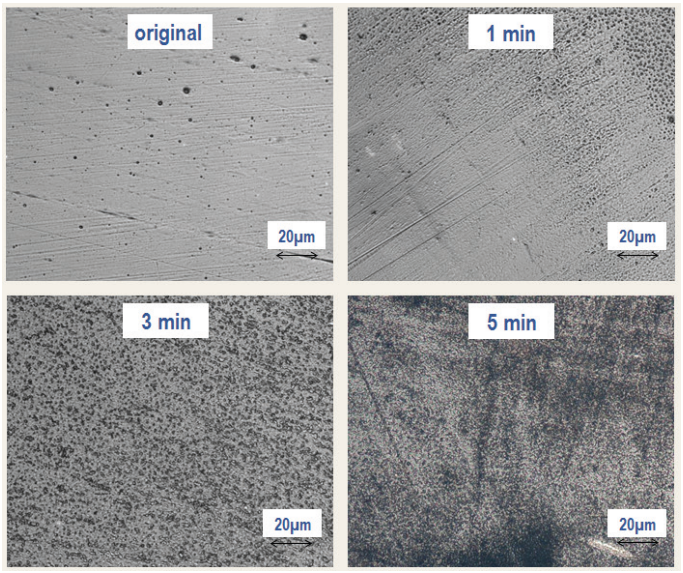

Fig. 2. Micrographs of PLA films after different DBD exposure times

Fig. 3. shows that the contact angle decreases from 55° to 43.5° at 1 minute, then the angle begins to increase; tends to the initial value of the contact angle (55°). This can be caused by the processes of degradation and chemical cross-linking in the surface layer of PLA during the DBD. High adhesion and proliferative activity of fibroblasts are provided by hydrophilic materials, so the chosen treatment time range is from 0.5 min to 3 min.

It is a known fact that the mechanical properties of polymer films change after the plasma treatment. The results are shown in Fig. 4.

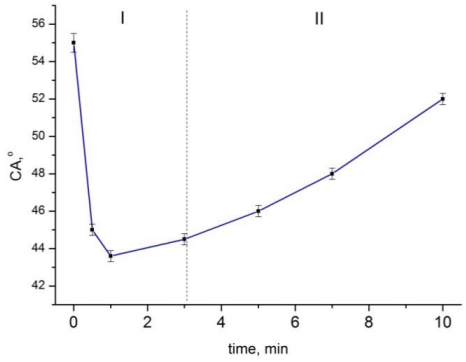

Fig. 3. Water contact angle (WCA) of the PLA samples versus DBD exposure time
Area I refers to a decrease in the WCA, area II refers to an increase in the WCA
on the treated surface of the PLA film

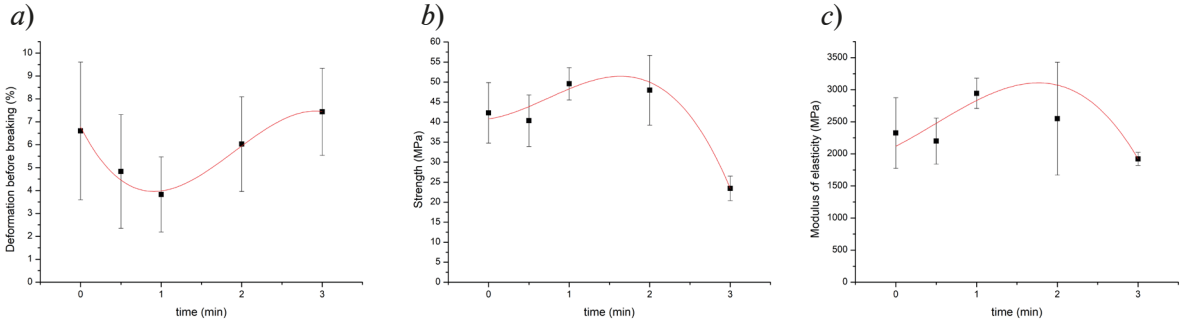

Fig. 4. Mechanical properties of the original and modified PLA films
Deformation before breaking (*a*), strength (*b*), modulus of elasticity (*c*)

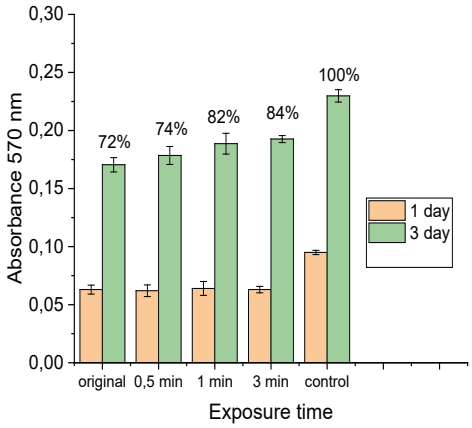

Fig. 5. Cell viability of human dermal fibroblasts cultivated
on the surface of PLA films for 1 and 3 days

The plot suggests that after 1 minute of DBD exposure, the elongation of PLA decreases to 4 ± 1.5%, the mechanical strength and the modulus of elasticity increase. This indicates the modification of films in DBD.

To analyze the biological properties of PLA films *in vitro*, a culture of human dermal fibroblasts was used. After 1 day of cell culture on the surface of films and culture plastic (control sample), cell adhesion was analyzed using a MTT test. It has been shown that all samples of PLA films have appropriate biocompatibility to maintain cell adhesion. Modification of the PLA matrix surface does not cause significant changes in the level of cell adhesion (Fig. 5). After 3 days of cell culture on the surface of PLA and culture plastic, a MTT test was used to determine the optimal treatment properties for maintaining an even distribution of cells on the surface of the material and a high level of their proliferative activity. The test showed that the optimal treatment time is in range from 1 min to 3 min.

Conclusion

The results of the study show that PLA films can be modified in DBD to increase the proliferative activity of fibroblasts. When the DBD exposure time is in range from 1 to 3 minutes with energy density of partially discharges about 8.6 J/cm^2, cell viability can be increased by 10–12% compared to an untreated PLA film. This result appears to be caused by a hydrophilic surface (WCA 44°) and a homogeneous rough relief of the treated PLA films.

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