Surface modification of polyimide film in the barrier discharge for cellular technologies

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Abstract. In this work, the surface of polyimide films of PMDA-ODA was modified using a barrier discharge in order to optimize their biological properties when interacting with a culture of human dermal fibroblasts. The optimal modes of processing films in a gas discharge, which allows to increase the proliferative activity of cells, are found.

1. Introduction

Currently, polyimides (PI) are used in medical technology for cardiovascular catheters, stent delivery devices, and drug delivery systems [1]. A distinctive feature of PI materials is the high stability of mechanical, electrical, and thermal properties in a wide temperature range from -200 to +400°C. Polyimide is a biocompatible material [2]. However, the biocompatibility will depend on the type of solvent used to produce the material (film, fiber, plastic) based on it, as well as the chemical structure of the PI macromolecule and its supramolecular organization.

As for any polymer material [3], the hydrophilicity of the surface of the PI, characterized, for example, by the value of the contact angle, should have a great influence on its biological effects. Increased cell adhesion to the polymer material is usually achieved in the case of its hydrophilic surface [4], and the hydrophobic surface of the polymer maintains a high level of protein adsorption on it [5]. On the other hand, increased moisture absorption can negatively affect the mechanical characteristics of the PI in the long term.

Polyimide materials intended for cell or tissue culture require a surface with a higher hydrophilicity, which in the case of polymers of this type can be achieved by laser or plasma treatment [6], as well as chemical modification of the PI surface [7].

The aim of this work was to create an optimal balance of the physical and chemical properties of the modified material for the specific regulation of cell behavior in accordance with the tasks of their cultivation and tissue engineering technologies due to the effect of a dielectric barrier discharge in the air on the surface of the polyimide film PMDA-ODA.
2. Materials and Methods
Films of polyimide PMDA-ODA were selected as the objects of research. Polyimide films were obtained by two-stage thermal imidization of a prepolymer-polyamide acid cast on glass substrates by irrigation. Polyamide acid was synthesized in an amide solvent by polycondensation of equimolar amounts of the initial monomers: diamine diphenyl ether and pyromellitate dianhydride. The structural formula of polyamide poly(-4,4'-oxidiphenylene) pyromellitimidie is shown in Figure 1.

![Figure 1. The chemical formula of polyimide PMDA-ODA.](image)

A dielectric barrier discharge (DBD) is an electrical discharge between two electrodes separated by an insulating dielectric barrier. The main factors leading to the modification of the film are bombardment with charged particles (ions and electrons) and oxidative processes. In the process of film modification, physical and chemical changes of the film surface occur, functional groups (-O, -CO) and cross-links appear.

The DBD was created in an ionization cell, which consists of ceramic plates with electrodes separated by an air medium (1 mm thick). For sufficient oxygen access, the ionization cell was blown by an air stream during the film processing. The gas discharge in such a cell develops evenly over the entire surface under the electrode. The PMDA-ODA film was placed in the gas-discharge gap. The source of the high-voltage signal was a line transformer TVS-110 using a ZVS driver in the primary winding. The schematic diagram of the DBD installation is shown in Figure 2.

![Figure 2. DBD circuit diagram: 1-ceramic plates, 2-electrodes, 3-sample, 4-high-voltage generator, 5-air fan.](image)

The value of the corona voltage was 2.6-2.7 kV with a frequency of 2.5 kHz, the use of high-frequency alternating voltage leads to an increase in the intensity of partial discharges. The processing time of PMDA-ODA films was welded from 10 to 600 seconds.

The change in the surface relief of PMDA-ODA films after their modification in the barrier discharge was determined using the ZEISS Axio Scope.A1 microscope.
The edge angle of contact of the PMDA-ODA films was determined by the lying drop method on a DSA30 device, Kruss (Germany). Distilled water and hexadecane were used as the wetting fluid. The free energy of the surface of these films was calculated using the extended Fock method [8], which uses the values of the contact angle of a drop lying on a solid surface for two liquids: water and hexadecane.

The energies were calculated in accordance with the following equations:

\[
\gamma_I'(1 + \cos \theta) = 2 \cdot \sqrt{\gamma_I^d \cdot \gamma_I^d} + 2 \cdot \sqrt{\gamma_I^p \cdot \gamma_I^p}
\]

\[
\gamma_s = \gamma_s^d + \gamma_s^p
\]

where, \( \gamma_I^d, \gamma_I^p, \gamma_I \) are the dispersion, polar, and total surface energies of the wetting liquid, respectively, mJ/m²; \( \gamma_s^d, \gamma_s^p, \gamma_s \) are the dispersion, polar, and total surface energies of the polymer surface, respectively, mJ/m².

To analyze the biocompatibility of materials in in vitro studies, a strain of skin fibroblasts from a conditionally healthy donor (collection of cell cultures of the Institute of Cytology of the Russian Academy of Sciences) was used. The cells were cultured in a complete culture medium in a CO₂ incubator (Thermo Fisher Scientific, USA) at a temperature of 37°C, a CO₂ 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, an MTT test was used. To do this, the growth 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 which 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 living cells was estimated by the optical density.

3. Results

The effect of the processing time in DBD on the microrelief of PMDA-ODA films was recorded using a light microscope; micrographs are shown in Figure 3.

As can be seen from Figure 3, as the exposure time in the barrier discharge increases, the relief of the films changes significantly. The density and depth of defects formed on the surface increase with the processing time.

Figure 3. Micrographs of PMDA-ODA films after different exposure times of the barrier discharge.
The measurement results showed (Figure 4) that an increase in the free energy of the surface and a decrease in the edge contact angle of contact of PMD-ODA films occurs after the first 60 seconds of their processing in DBD and indicates a significant increase in the hydrophilicity of PMD-ODA films. An increase in the free surface energy (from 20 to 70 mJ/ m²) determines an increase in the number of functional groups (-O, -CO). Further processing time of PMD-ODA films, more than 60 seconds, does not lead to a noticeable increase in the hydrophilicity of their surface.

![Figure 4](image-url)

**Figure 4.** Dependence of the free energy of the surface and the edge angle of contact of the PMD-ODA film on the time of its processing in the DBD. 1-Free surface energy, 2-Contact angle with water, 3-Contact angle with hexadecane.

To analyze the biological properties of experimental samples of PMD-ODA films in *in vitro* studies, a culture of human dermal fibroblasts was used. After 1 day of cell culture on the surface of the films and culture plastic, which was used as a control, the cell adhesion was analyzed using the MTT test. It was shown that all samples of PMD-ODA films have biocompatibility properties to maintain cell adhesion. Modification of the surface of PI matrices does not cause significant changes in the level of cell adhesion (Figure 5). It was shown using the MTT test that after 4 days of cell culture, optimal properties are created on the surface of PI matrices and culture plastic to maintain a uniform distribution of cells on the surface of the material and a high level of their proliferative activity. After processing for 3 minutes, the polyimide film had a high biocompatibility (Figure 5).
Figure 5. MTT test with human dermal fibroblasts cultured on the surface of polyimide films for 1 and 4 days. The control cells were grown on the culture plastic.

4. Conclusion
A dielectric barrier discharge at atmospheric pressure in an air medium was used to modify the surface of PMDA-ODA films. The frequency of the partial discharges and their amplitude were stable due to the effect of the air flow in the gas-discharge gap. It was shown that the treated PMDA-ODA films have a high free surface energy (69.5 mJ / m²) and a low contact angle (17.5°). It was found that 3 minutes of treatment in DBD creates an optimal microrelief of the film surface to improve cell regeneration. At 3 minutes of plasma treatment, the proliferative activity of cells increases by 15% compared to the original film. It is most likely that after treatment, oxygen functional groups appear on the surface, which actively interacts with the cells and improve their proliferation.

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