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DEPOSITION OF POLYMERIC THIN FILMS FROM PROPANE-BUTANE IN ATMOSPHERIC PRESSURE DISCHARGE

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Abstract. In our experimental work we prepared thin films via plasma polymerisation and characterized them. These films were prepared on a glass substrate in the dielectric barrier discharge (BDB). The wettability of the deposited layers was determined by measuring of the contact angle and the free surface energy of the layers using the SEE system apparatus. The atomic composition and the surface morphology of the thin layers was characterized by scanning electron microscopy (SEM). Performed biological tests of biocompatibility and antibacterial properties provided information on the viability of the cells on the layers and their antibacterial effects.

Keywords: plasma polymerization, DBD, thin films, propane-butane, biocompatibility.

1. Introduction

Plasma polymerization is the process of creating a highly-branched polymer by plasma-initiated polymerization of the gas precursor. Typically, plasma polymer is created as a thin layer consisting of short chains with random organization and a high degree of crosslinking. The formation of thin layers and coatings on the surface of a material makes it possible to significantly change its properties without affecting the volume, or to combine the properties of two or more materials. Thin films are widely used as functional, reinforcing, reflective, conductive and dielectric materials in the formation of contacts, the production of printed circuit boards, integrated circuit elements in microelectronics, the creation of light filters and the basic elements of optoelectronics. Thanks to intensive experimental and theoretical research in the development of thin film technology, significant progress has been made in recent years [1–3]. Based on the set conditions, the properties of the selected substrate and the selected configuration of the electrodes, it is possible to deposit either hydrophobic or hydrophilic layers [1]. The so-called (highly) hydrophilic layers can be prepared by the plasma enhanced chemical vapor deposition (PECVD) [4]. These layers have found their application, for example, in biomedical applications as an environment for endothelial cell growth [5, 6] or in the modification of polyethylene terephthalate (PET) [3]. In this paper, we would like to introduce a method for the fast and permanent surface hydrophilization of a surface. The method is based on the plasma deposition of plasma-polymeric nanolayers constituted from basic elements only (C, H, N, O).

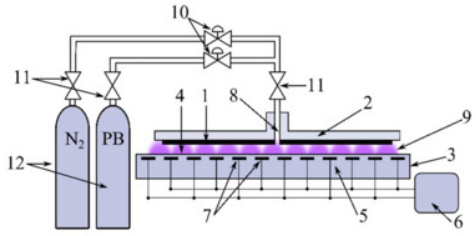

Figure 1. Schematic representation of the used deposition apparatus : 1) sample 2) sample holder 3) DCSBD electrode 4) plasma layer 5) dielectric layer 6) HV power saupply 7) strip electrodes 8) gas inflow 9) gas outflow 10) gas flow controller 11) shut off valve 12) gas cylinders [7].

2. Experimental set-up

The schematic drawing of experimental set-up is shown in Figure 1. The deposition apparatus consists of a reactor (customer made), a high voltage AC generator (Lifetech, Brno, Czech Republic) using sine-wave high voltage with a frequency of 30 μkHz and gas cylinders with a reaction mixture of gases. Plasma polymerization was performed in the reactor on the substrate from the reaction gas mixture with surface DBD. Glass plates (soda-lime glass, 150×100 mm, thickness 1.1 mm) were used as substrates for deposition. The glass substrate was purified in a mixture of cyclohexane and isopropyl alcohol (1:1) and thoroughly dried before deposition. The electrode system consists of 11 cylindrical surface electrodes made of brass with a length of 10.4 cm and a diameter of 1 cm, between which there are gaps with a width of 2 mm. On one side of the dielectric layer there are 11 surface electrodes, on the opposite side there is a flat grounded electrode. The main dielectric component

is aluminium (III) oxide, limiting the electric current in the space between the electrodes. A substrate was inserted between the electrodes and the dielectric, on which layers were deposited. The grounded electrode is connected to a water cooling apparatus in which was water with a temperature of approximately 20 °C flows. The cooling of the lower electrode is necessary in order to avoid overheating of the deposited layers and thus to the destruction of the formed bonds and loss of the required properties.

The reaction gas mixture for deposition consisted of nitrogen and propane-butane (P-B). The mixture contained 84% propane, 15% butane and 1% C_2 and C_5 hydrocarbons. The volume concentration of propan-butan was set at 0.4, 0.7, 1.0, 1.3, 1.6 and 1.9%, and a total gas flow of 6.5 l min^{-1} was kept in all experiments.

The wettability of sample surfaces was determined by measuring the static contact angle using the sessile drop method. Sessile drop of a volume of 1 µl was dropped onto the measured surface and analyzed using See System E (Advex Instruments, Brno, Czech Republic). The contact angles of three standard liquids (deionized water, diiodomethane and ethylenglykol) were measured and the surface free energy was calculated by the Owens, Wendt, Rabel and Kaelble (OWRK) model [8].

The MIRA3 TESCAN scanning electron microscope with Energy Dispersive Spectroscopy (SEM-EDS) was used to determine the elemental composition and morphological properties of the prepared layers.

Biocompatibility tests were made in vitro using rat vascular smooth muscle cells (VSMC) cultured in Dulbec's modified Eagle's medium (DMEM; High glucose, Gibco, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Pure substrate and the samples were cut and placed in cell culture wells. Three wells were reserved for each sample and substrate. Cells were seeded at a concentration of $2 \cdot 10^5$ cells per well of sample at the beginning of the experiments. Then 0.5 ml of nutrient medium was added to each well. The cells were cultured under constant incubation conditions (temperature 37 °C, 5% CO_2 and 95% humidity) in culture medium for 72 hours. After culturing, the sample wells were illuminated and taken with an Olympus inverted light microscope.

The method of obtaining the relative amount of adenosine triphosphate (ATP) was performed to determine cell viability. The method is based on the chemiluminescence of ATP from a lysate of cells cultured on modified surfaces. Two sets of samples were measured to compare the number of cultured cells. In the first case were counted bacteria cells, which grown directly on the modified surface. In the second case the cells in the wells around the substrate were counted. At the beginning of the measurement, the culture medium was aspirated and the coated substrate was transferred to new wells. The wells of the slides and the wells of the cultured cells at the bottom were washed with phosphate-buffered saline (PBS) and then 200 µl of Somatic cell ATP releasing reagent (Sigma Aldrich) was added to each well and left on a shaker for 10 minutes. After 10 minutes, 20 µl of lysate was collected and this amount was then mixed with 20 µl of ATP mix solution (191013 Cot, BioThema, Handen, Sweden) containing a luciferase-luciferin mixture. To avoid degradation of the active compounds in the reaction solutions, the samples were measured as quickly as possible on a LMT 01 luminometer (Immunotech, Monrovia, USA) and the number of viable cells was determined using the Microwin 2000 software.

Antibacterial tests were performed with bacterial culture of Staphylococcus epidermidis (CCM 4418, CCM – Czech Collection of Microorganisms) to prove antibacterial effects. This bacterial culture was grown into commercial BHI medium (Brain Heart Infusion Broth, HiMedia, Mumbai, India). The cultivation was carried out under standard conditions at a culture temperature of 37 °C and for 24 hours. Next, the bacterial culture was diluted with new sterile medium to $1 \cdot 10^8$ CFU/ml based on turbidity (NanoPhotometer TMP300, Implen, Munich, Germany). Subsequently, a 0.5 ml suspension of Staphyloccocus epidermidis was added to each sample. The bacteria were incubated on the surface of the layers for 24 hours to allow biofilm formation. The results were evaluated using optical microscopy (Intraco Micro LM 666 PC with Dino-Capture 2.0 software, Tachlovice, Czech Republic).

3. Results and discussion

3.1. Free surface energy

Free surface energy was determined by measuring the contact angle of the tested liquids on the surface of (highly) hydrophilic layers and substrate. Table 1 shows the contact angles of the liquids and the free surface energies of the individual layers. The total free surface energy of the hydrophilic layers increased significantly compared to the surface energy of pure glass. The total value of energy consists of the Lifshitz-Van der Waals interaction (LW) and acid-base interaction (AB) values. LW interactions are non-covalent and non-electrostatic in nature between molecules and have their origin in the interaction of randomly arranged dipoles. AB interactions describe interactions between biopolymers and low-energy surfaces and are based on Lewis's theory of generalized acids (electron acceptors) and bases (electron donors) [8]. As the concentration of P-B increases, the value of the acid-base interaction increases. It can be seen from the measured contact angles that all layers are (highly)hydrophilic with an uncertainty of about 15%, so that the contact angle decreased for each of them and the surface energy increased. The contact angle of the water test liquid is less than 10° and the free surface energy is around 41.60 to 59.24 mJ/m^2.

PB concentration [%]	contact angle [°]			free surface energy [mJ/m^2]		
	water	diiodomethane	ethylene glycol	overall	gLW	gAB
substrate	28.64	53.45	30.45	33.52	32.33	1.19
0.4	11.78	44.79	13.94	39.31	37.12	2.18
0.7	10.49	30.41	11.35	55.02	44.05	10.97
1.0	7.90	25.22	8.58	59.24	46.07	13.17
1.9	9.79	35.27	23.58	58.01	41.90	16.10

Table 1. Contact angles of tested liquids and free surface energy of deposited layers.

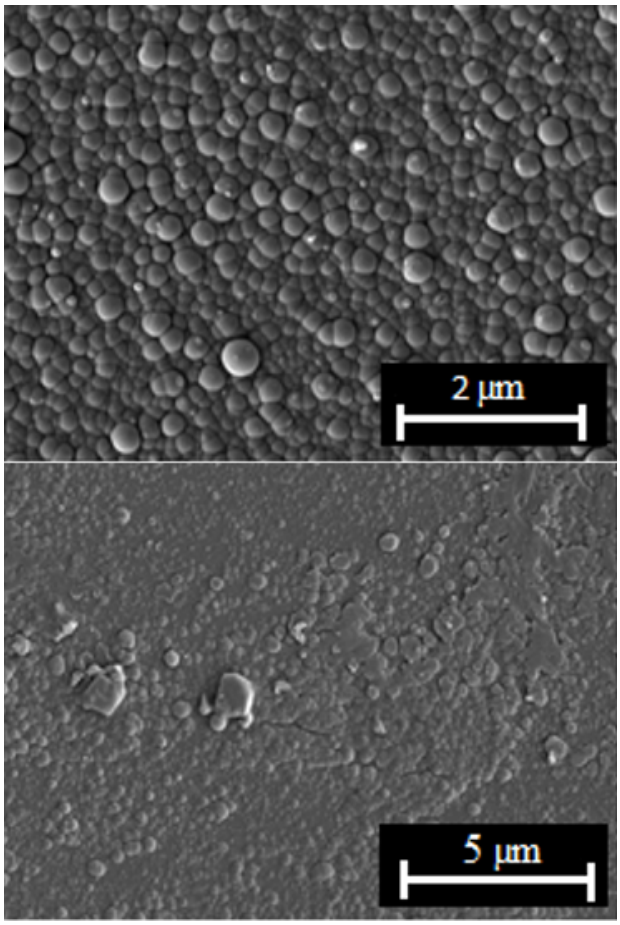

Figure 2. Image of the polymer surface of the layer 0.7% P-B and 1% P-B at magnifications of 2 and 5 µm.

3.2. Scanning Electron Microscopy with Energy Dispersive Spectroscopy

The surfaces of the samples at several selected concentrations were imaged using a microscope and then detected. It can be seen in Figure 2 that the surface is not homogeneous and corresponds to the polymer structure.

Then the atomic composition of the layer surfaces was determined by the EDS detector, which allows to perform elemental analysis based on the analysis of the energy emission of the X-ray spectrum. Table 2 shows the atomic composition of the layers at different P-B concentrations. The percentage of nitrogen and carbon increases with the concentration of the propane-butane reaction gas. In contrast, the amount of oxygen decreases with increasing concentration. This can be explained by the formation of new functional groups on the surface when passing a more concentrated gas, such as an increase in the number of cyan groups (CN).

element	PB concentration [%]		
	0.7	1.0	1.3
N	29.4	32.0	33.3
C	48.7	53.5	61.1
O	22.0	14.5	5.9

Table 2. Atomic composition [%] of the surface of the layers depending on the PB concentration.

3.3. Biocompatibility tests

Biocompatibility tests were performed in vitro using rat VSMC cultured in DMEM. The pictures form inverted light microscope are in Figure 3. The results of the measurements are shown in Figure 4. The figure shows a comparison of the relative number of cells, which was determined by the ratio to the original number of cells - value 1, or 100%. The graph shows that samples with a propane butane concentration of 0.7 and 1% do not support cell proliferation and, conversely, stop it and behave as toxic to cells. The given results are unexpected and can be explained, among other things, by mechanical microdamage of the superimposed layers. The measured values in the wells without substrate are in all cases greater than the unmeasured values with it. The graph also shows that the increasing number of ATP cells increases with increasing P-B concentration. It could be said that layers not deposited at a concentration of 1.9% P-B promote cell viability.

3.4. Antibacterial tests

For antibacterial tests was chosen bacterial culture of *Staphyloccocus epidermidis* (CCM 4418). This microorganism occurs on the surface of the skin and mucous membranes of any healthy person and does not cause any damage to health. Prior to culturing,

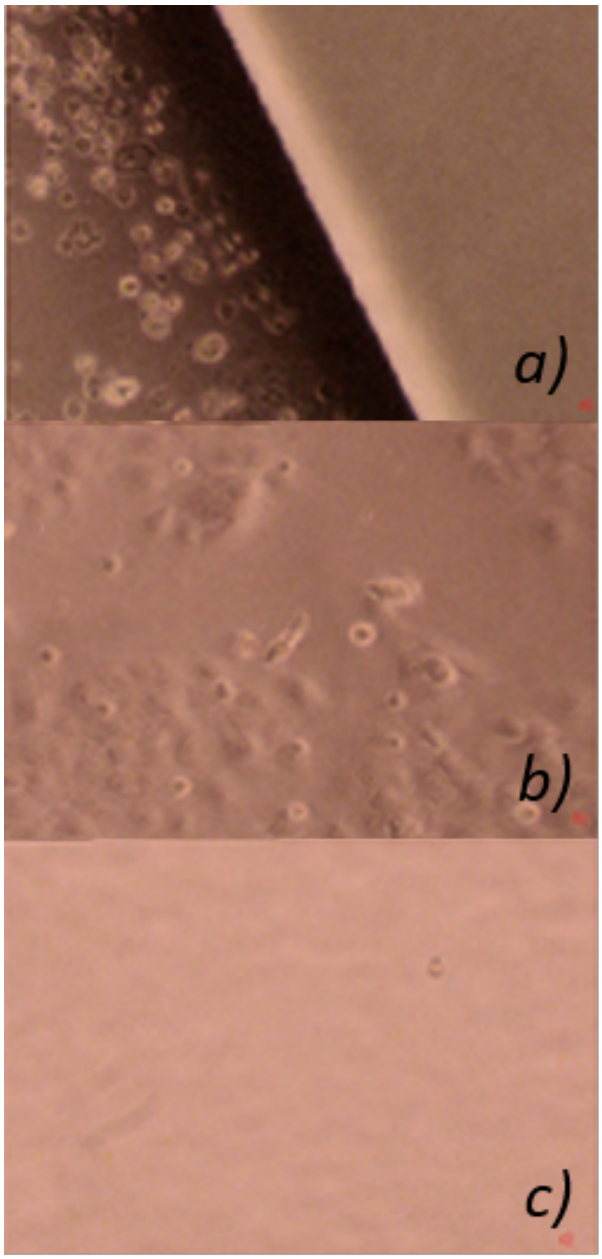

Figure 3. Image a) interface between layers b) pure substrate with VSMCs cultured on them and c) deposited layers at a concentration of 1.3% P-B.

samples of the layers deposited at various P-B concentrations were washed once and then twice with sterile Milli-Q water to ensure layer stability. S. epidermidis bacterial culture was grown in BHI liquid medium, which is based on infusion of heart and brain tissue. Each sample before and after rinsing was photographed at least 12 times to determine the more accurate number of cells per layer. Based on the images of the deposited layers, a graph of the amount of cultured bacteria on the P-B concentration used during deposition was constructed (Figure 5). The graph shows that as the concentration of P-B increases, the number of bacteria on the surface of the layer's increases. In addition, layers with a P-B concentration of 1.3, 1.6 and 1.9% significantly support cell growth. As already found in the performed biocompatibility tests, the layers with a concentration of 0.7 and 1% are cytotoxic to the cells.

4. Conclusions

The main goal of this work was the preparation of layers by plasma polymerization and their characterization. The layers should show increased hydrophilicity, biocompatibility and support the growth of bacterial cells. Pure glass was chosen as the substrate during the deposition and the polymerization took place in a mixture of propane-butane reaction gas and nitrogen. The deposition took place at atmospheric pressure and at a constant temperature. In addition, certain propane butane concentration flows were set for each measurement. Subsequently, the prepared layers were characterized using physic-chemical diagnostics. The first diagnosis was the determination of free surface energy based on the measurement of contact angles of liquids with different viscosities. It was proved that each prepared layer was hydrophilic or (highly)hydrophilic. Another task was to determine the elemental composition of the surface of the layers using scanning electron microscopy (SEM). The largest percentage was for carbon (about 50%), then for nitrogen (about 30%), and finally for oxygen (about 12%). The percentage of nitrogen and carbon increases with the concentration of the propane-butane reaction gas. In contrast, the amount of oxygen decreases with increasing concentration. The thickness of the polymer layer was about 106.45 nm. Biological tests for biocompatibility and antibacterial properties were also performed. Experiments were performed in vitro using rat VSMC vascular smooth muscle cells. To determine cell viability, a relative amount method was performed. The results of this test are inconclusive, because the layers superimposed at P-B concentrations of 0.7 and 1% are rather toxic and therefore do not support cell proliferation, but on the contrary stop it. The other samples promoted cell viability and the best result was at a concentration of 1.9%, where the viability was around 120%. Antibacterial experiments with bacterial culture of *Staphyloccocus epidermidis* (CCM 4418) were performed and some results were unexpected because the lower concentration layers again showed toxic properties. In contrast, layers superimposed at a higher P-B concentration of 1.3; 1.6 and 1.9% supported bacterial growth, which was the goal of the test.

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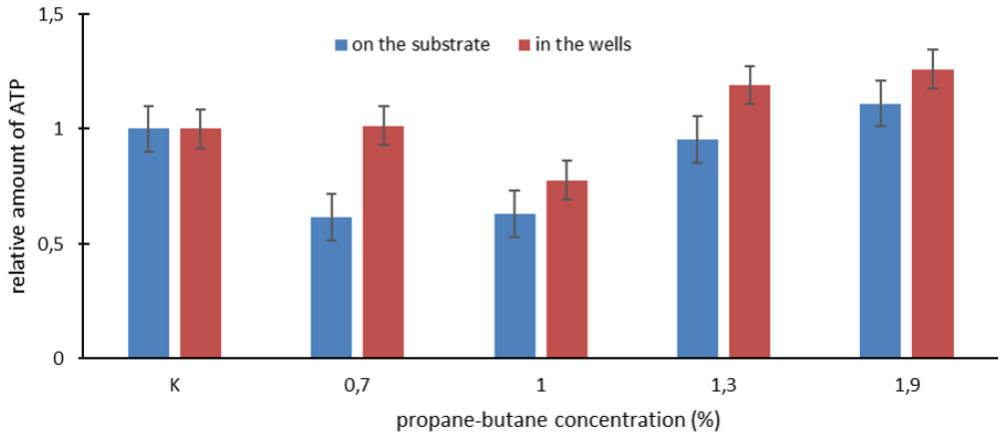

Figure 4. Relative amount of ATP in cells cultured on a layered substrate at different concentrations of P-B and without substrate-glass (K).

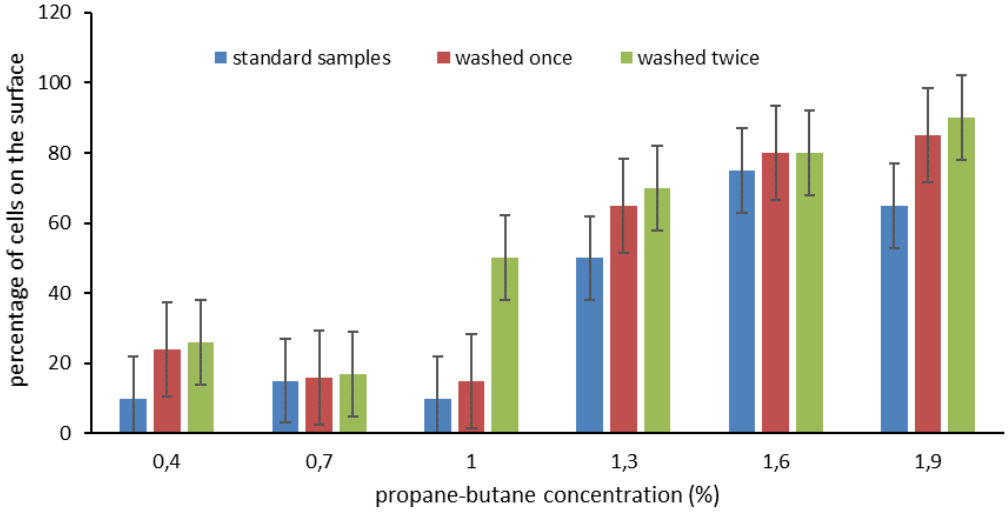

Figure 5. Percentage of S. epidermidis cells cultured on sample surfaces superimposed at various concentrations of P-B.

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