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# Plasma treatment of the surface of poly(hydroxybutyrate) foil and non-woven fabric and assessment of the biological properties

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ABSTRACT

This paper deals with the poly(hydroxybutyrate) (PHB, foil and fabric) surface modification, characterization and the view of its possible application. The influence of Ar plasma treatment on surface polarity was studied. The changes of the surface parameters were determined immediately after treatment and after annealing related to plasma power, treatment time and heating. These surface-induced differences were studied by different analytic methods: polarity (wettability) was studied by contact angle measurement and surface energy calculation, the surface morphology analysis was done by atomic force microscopy and for determination of chemical composition of surface layer the XPS analysis was used. It was found that after plasma treatment the surface energy of both PHB foil and non-woven fabric significantly increased. PHB non-woven fabric exhibited almost immeasurable contact angle after the plasma treatment. The wettability of both types of PHB substrates after heating was completely inversed. The significant change in roughness of PHB foil was found. After the plasma treatment, the material ablation was determined, which was also connected with surface chemical changes, thus the surface was found to be corrugated. The heating procedure induced "little" crystallites on the surface. Positive effect of PHB foil modifications on surface biocompatibility was confirmed. The biocompatibility was also preserved when thermal stress was applied. Silver nanolayer sputtered on PHB fabric surface induced strong antimicrobial properties.



## 1. Introduction

Surface modification is a process that alters surface of a material resulting in changes in physical, chemical and biological properties [1]. Many techniques of surface treatment have been developed to improve polymer properties (e.g. degree of crystallinity, wettability, adsorption, printability, and chemical reactivity [2–4]).

The principle of wet methods is a direct contact of a polymer [5] with a reagent to give reactive functional groups, which affect the polymer properties. The classic approach for surface treatment does not require any special equipment, thus it can be easily performed in almost every laboratory. The degree of functionalization of a material surface may be reproducible among polymers having different molecular weight, tacticity or crystallinity. Many of these techniques also require a longer treatment time in the concentrated solutions. For these reasons some of the wet methods may not suitable for a large number of industrial applications [4]. Another method for surface treatment is polymer grafting. The aim of grafting is the covalent attachment of suitable groups or macromolecules on the substrate surface [6–10]. Very often the grafting of thiols is applied, where the thiol group (—SH) improves adhesion of gold nanoparticles on a polymer surface [11]. The method of immobilization of organosilanes, which was originally developed as a means for connecting the organic polymer to an inorganic substrate (e.g. to promote adhesion during the development of fiberglass) can be also applied on different solid substrates [4].

The major advantage of plasma treatment is its simplicity, reliability and affordability. Furthermore, it was shown that it is applicable for destroying bacterial cells, which allows in situ biomaterial sterilization [12,13]. Plasma in contact with the solid material changes its surface properties (e.g. wetting, metal adhesion, dye ability, chemical inertness, lubricity, and biocompatibility) [14,15]. Under changed conditions, such as temperature, pressure, power, gas and substrate type, either the surface functionalization, co-deposition, ablation or cross-linking takes place. Functionalization can be performed also as grafting of bioactive molecules, e.g. fullerenes [16,17] or carbon particles [18,19]. Most of the modifications are performed using low temperature or "cold" plasma. When we speak about plasma, we mean the highly ionized gas consisting of neutral and charged particles (ions, radicals, excited molecules, electrons), wherein the total charge remains neutral [20]. Plasma can provide modification of the upper layer of polymer surface without using solvent or generating chemical waste and with less degradation and roughening of the material than many wet chemical treatments [4]. This procedure enables the modification of heat-sensitive materials,

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which are e.g. polymers [21]. This type of plasma can be generated in many ways, for example by corona discharge treatment, UV-radiation, X-ray beam, and gamma-irradiation. The most common applications of plasma treatments are surface cleaning or etching [22], to increase the wettability and adhesion of polymers [23] and more recently, to improve cell attachment in tissue culture studies or to alter surface protein interactions [24,25]. Functional groups and cross-links are introduced at polymer surface by reaction of gas-phase and surface species. Improving adhesion characteristics, increasing hydrophobicity, introducing special functional groups at a surface, or modifying the surface morphology are examples for the purposes of these surface treatments [26–28]. A broad range of functional groups can be introduced at the surface by variation of the gas used.

Modified or grafted surfaces may be examined as potential cell carriers for tissue engineering applications [14–19] or anti-bacterial surfaces. Tissue engineering scaffolds [29–31] are able to establish three-dimensional environments for propagation of cells and specific signal molecules that can mimic native tissue environment. Such scaffolds can be natural, synthetic or a hybrid of both. The use of signal molecules has the potential to increase scaffold effectiveness, by directing the phenotypes of contained cells, as well as indirectly that of surrounding cells. Living host cells can also migrate into an implanted scaffold containing only biofunctionalized spaces [32], or can be expanded and allowed to associate with scaffolds as substrates in tissue culture before implantation [32]. Polymeric biomaterials are currently dominated by polyesters such as poly(lactic acid) (PLA) [33], poly(hydroxybutyrate) [34], poly(glycolic acid) (PGA) [35], polycaprolactone (PCL) [36] and their blends or copolymers or fibrous polymers such as cellulose or chitosan [37]. Polyester [38] is a category of polymers that contain the ester functional group in their main chain. Esters are chemical compounds derived from a carboxylic acid (—COOH group) and a hydroxyl (—OH) compound, usually an alcohol. Although these biomaterials have been well characterized and fabricated to match the biochemical properties of soft tissues, there is generally a lack of mechanical compatibility between polymer implants and living tissues. An interesting study has been also published by Bismarck et al. [39], in which biopolymer cellulose nanofibers were modified by etherification reaction. Still only a few studies have been reported on biopolymer poly(hydroxybutyrate) [40,41], especially in the form of non-woven fabric, i.e. with no poly(hydroxyvalerate).

This paper deals with the poly(hydroxybutyrate) foil and non-woven fabric. The influence of plasma treatment on substrate polarity and surface energy was studied. The effect was examined immediately after the treatment and then after thermal annealing related to plasma power, exposure time and annealing time and temperature. Polarity of the substrates was studied by contact angle measurement and surface energy calculation. For the surface morphology analysis the AFM method was used. For the determination of chemical composition of surface layer, the XPS analysis was used. Effect of PHB foil treatment on cell adhesion and proliferation after plasma treatment and annealing was determined. Anti-microbial potency of silver sputtered PHB non-woven fabric was determined using *Escherichia coli* as a model organism.

## 2. Material and methods

### 2.1. Materials and modification

Poly(hydroxybutyrate) (PHB, with 8% poly(hydroxyvalerate), density 1.25 g $cm^{-3}$, upper working temperature 95 °C) in the form of 50 μm thick foils (supplied Goodfellow, Ltd.) was used. As the second substrate, we used a biopolymer in the form of non-woven fabric PHB (density 1.25 g $cm^{-3}$, Goodfellow Ltd.).

The samples were modified in diode plasma discharge on Balzers SCD 050 device for 0–240 s, using DC Ar plasma (the gas purity was 99.997%, the power of 3 and 8 W). Process parameters were: Ar flow of 0.3 l $s^{-1}$, Ar pressure of 10 Pa, electrode area of 48 $cm^2$, the inter-electrode distance of 50 mm, and chamber volume of 1000 $cm^3$.

Thermal treatment of the polymers was accomplished in thermostat BINDER. The samples were heated at 75 or 100 °C (upper working temperature of PHB). The pristine and modified samples (immediately after plasma treatment) were heated for different time intervals and then they were cooled down to room temperature.

We used BAL-TEC SCD 050 for silver deposition in regime sputtering. The distance of electrodes of 5 cm, Ar pressure of 5 Pa, and sputtering current of 20 mA were used. Deposited average thickness was approx. 3 nm. Ag sputtering target (Safina s.r.o., Czech Republic) with purity > 99.99% was used for the deposition procedure.

### 2.2. Measurement techniques

#### 2.2.1. Wettability

Contact angle was determined by goniometry with static water drop method. The measurements of the advancing water contact angle (error ± 5%) were performed using distilled water on nine different

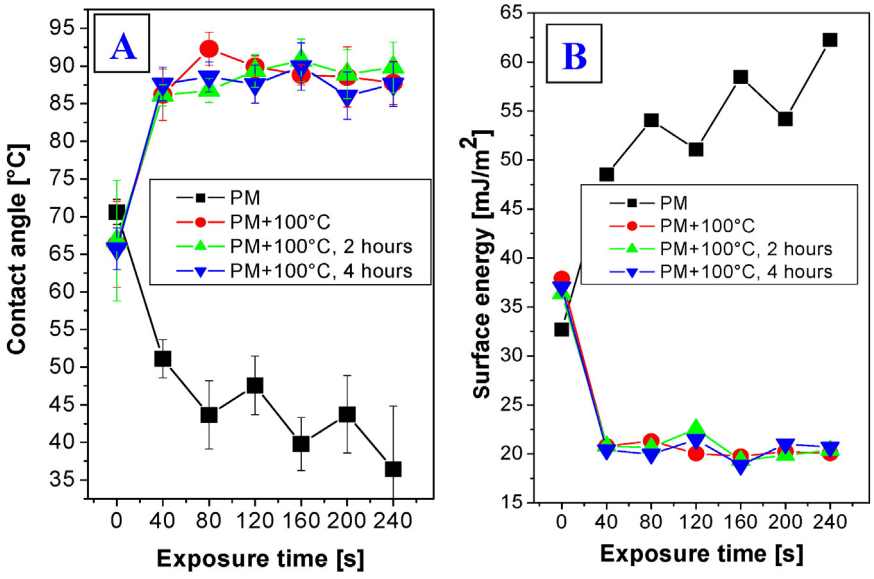


**Fig. 1.** Dependence of the contact angle between PHB foil surface and water drop on the length of plasma (8 W) modification (A), plasma treated samples (PM) were consequently annealed for 1 h at 100 °C (PM + 100 °C) and further aged for 2 h (PM + 100 °C, 2 hours) and 4 h (PM + 100 °C, 4 hours). The same dependence is introduced for surface free energy (B).