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Plasma induced cytocompatibility of stabilized poly-L-lactic acid doped with graphene nanoplatelets

P. Slepička[a,*], N. Slepičková Kasálková[a], A. Pinkner[a], P. Sajdl[b], Z. Kolská[c], V. Švorčík[a]

[a] Department of Solid State Engineering, University of Chemistry and Technology Prague, 166 28 Prague, Czech Republic
[b] Department of Power Engineering, University of Chemistry and Technology Prague, 166 28 Prague, Czech Republic
[c] Faculty of Science, University of J. E. Purkyně, 40096 Ústí nad Labem, Czech Republic

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ABSTRACT

The process of preparation, optimization and volume doping of thin poly-L-lactide acid foils is described in this paper. The goal of the doping is to improve overall properties as well as surface nanostructures construction by consequent plasma treatment. The next step of surface enhancement of graphene doped foils involved argon plasma treatment or combination of plasma and heat treatment. The certain combination of plasma treatment and graphene nanoplatelets doping increased the cytocompatibility of PLLA surface for vascular smooth muscle cells significantly. This result is also important for possible electrical stimulation of neuron or other cell types, which would proliferate on biopolymer and for construction of several types of biopolymer sensors based on conductivity measurement, yet prepared on eco-friendly biopolymer basis.

1. Introduction

Polylactic-L-lactic acid (PLLA) is a biodegradable aliphatic polyester that is increasingly popular with properties that enable its use in healthcare [1]. The properties of PLLA are influenced by many factors that can be modified during production. For example, crystallinity (the ratio of crystalline and amorphous phase) has a major effect on hardness and tensile strength. PLLAs with a high L-isomer content (> 90%) are crystalline, whereas with other content they are amorphous. This also affects the melting temperature and the glass transition temperature, which increase with the amount of PLLA. Crystallinity is affected by special catalysts added during production [2]. The PLLA has also a great potential in the food industry as a packaging material. For these purposes, other polymers and antioxidants and plasticizers may be added, and these components are used for improving of mechanical and barrier properties for gas [1,3]. Another sector benefiting from PLLA features is healthcare, as PLLA is naturally found in the human body, it is nontoxic and biodegradable. Also, the products of its disintegration are eliminated without difficulty from the body. To improve properties, it is often combined with other polymers (acid polyglycolic acid (PGA), hyaluronic acid (HA), etc.) [4]. Specific applications include implants - bone stabilization, a supporting structure for tissue growth, systems for targeted drug distribution or antibacterial surfaces [5].

PLLA is only exceptionally used in “pure” form. The most common is the use of copolymers, or the formation of composites with different (nano) particles and fibers [6]. Modern is the creation of so-called conductive polymeric nanocomposites that combine a nonconductive polymeric matrix with conductive nanoparticles. These composites can react to chemical, thermal, or mechanical stimuli by changing electrical properties, making them of interest in the manufacture of sensors [7]. Composites of PLLA and multi-wall carbon nanotubes (CNT), which served as a sensor for the detection of volatile organic solvents was already prepared [8]. The base for the sensor was constructed by spraying the solution of PLLA and CNT in chloroform onto the surface of the electrodes, after evaporation of the solvent, a polymer layer with a thickness of 1.5 μm and a CNT content of 2–3 wt% was formed [8]. The low humidity sensor was developed with PLLA and gold nanoparticles. Their composite mixture was used as a coating for quartz microwaves electrodes, a change in the quartz crystalline resonance frequency was detected that was higher in PLLA-Au coating than in PLLA or Au alone, which is attributable to a higher affinity for water vapour molecules [9].

The PLLA property, which can be interestingly used, is biodegradability. When a volume-doped layer is created that gradually breaks down and reveals built-in nanoparticles, the surface properties change, recent research on these dynamic surfaces worked with ZnO nanoparticles that were incorporated into the PLLA matrix [10]. Their potential use includes tissue engineering, as zinc is essential for cellular processes, and ZnO nanoparticles have antibacterial properties. The combination of sensor and medical applications can be so-called

* Corresponding author.
E-mail address: petr.slepicka@vscht.cz (P. Slepička).

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biodegradable sensors that serve their purpose for a short time (for example, during regeneration after an injury) and then decompose without the need for further surgery. They consist of two components - biodegradable polymeric matrix (e.g. PLLA) and biodegradable metal (e.g. Mg, Fe) [11]. The incorporating of such species into the polymer volume may be used instead of deposition of carbon based materials, e.g. fullerenes [12,13], carbon nanoparticles [14] or metal nanoparticles [15] on the polymer surface.

There are several approaches for nanolayer preparation, such as spin coating process [16], where we can estimate the layer thickness on the basis of rotates per minute, which allows us to prepare ultra thin conductive nanofilms [17]. Evaporation from the solution is one of the oldest techniques for the preparation of thin polymer films and, above all, in the continuous setting it is still being used. Methanol, acetone or water are often used in the production of biopolymer films [18]. The feed material in the form (e.g. powder, flakes or granules) is dissolved in a suitable solvent and consequently dried forming the layer. The technique of drop casting can be characterized as droplet drops of the solvent-polymer mixture to the stationary substrate. During its preparation, the solution "flows" over the surface of the substrate and evaporates to form the polymer layer, which can be enhanced to SVADC (substrate vibration-assisted drop casting) [19]. Changes in properties can be observed in surface morphology, decreasing roughness or in electrical conductivity improvement [19,20]. Several approaches were also used for the modification of biopolymer surface foils, based on grafting techniques [21], laser [22–24] or plasma treatment [25]. Graphene based composites can be also used for absorption of microwave radiation with possibility of development of high performance MAMs with small thickness, low density, wide bandwidth, and strong absorption [26]. Graphene-based materials and their composites were recently used for promising applications in wide range of fields such as electronics, biomedical aids, membranes, flexible wearable sensors and actuators, the summarizing review may be found in [27]. For the field of sensor applications the graphene based nanocomposites play an important role, recently was intensively studied e.g. engineering of nanomaterials-based biosensors for food safety detection [28] or electrochemical sensor and biosensor platforms based on advanced nanomaterials for biological and biomedical applications [29]. The porous polymers are of high importance, a very interesting papers focused on the preparation and characterization have been publihed by Bicmarck et al. [30,31].

This work is focused on preparation of bulk doped PLLA layers with graphene nanoplatelets (GNP) and the surface activation. We studied the impact of graphene doping on surface properties of studied samples (surface morphology, roughness, surface area, wettability) using different analytical methods and also influence on mechanical properties of prepared composites. Homogeneously doped PLLA with graphene nanoplatelets was consequently activated both with plasma and heat treatment. We have examined the surface physico-chemical changes of treated PLLA, consequently both the pristine doped and the plasma treated samples were chosen for experiments on cytocompatibility (adhesion and proliferation of vascular smooth muscle cells). Thus the high potential in electrical stimulation of different types of cells was uncovered and also mechanical stability revealed that the biosensors may be constructed on the basis of this research.

2. Material and methods

2.1. Material

The solvent casting method was used for the preparation of the GNP doped layers. The pristine polymer (PLLA, 50 μm thick, Goodfellow, UK) was dissolved in chloroform (stabilized with 1% ethanol, PENTA s.r.o.) in the appropriate amount according to the desired thickness of the layer. Evaporation of the solvent took place in the digester under laboratory conditions (temperature about 23 °C, atmospheric pressure) for 8 h. For better mixing and dissolution of the PLLA and the PLLA with GNPs, the solution was placed in the ultrasonic bath XUBA 1 (Grant, UK) for 300 s. The graphene nanoplatelets used were purchased from Goodfellow, UK. The GNPs were treated with argon discharge by the manufacturer to improve dispersion and solution interaction. The required amount of GNPs was weighed and corresponding solutions (0–20 wt%) were prepared.

2.2. Plasma treatment

Plasma treatment was performed on the BAL-TEC Sputter Coater SCD 050 (in the "etching" mode). The polymer foils were treated with argon plasma (10 Pa) by (i) 3 W (6 mA and 500 V) and (ii) 8 W (12 mA and 660 V) for 240 s.

2.3. Characterization

Contact angle was determined by goniometry with static water drop method. The measurements of water contact angle were performed using distilled water (6 different positions) using the Surface Energy Evaluation System (SEE System, Advex Instruments, Czech Republic).

Surface morphology was examined with atomic force microscope Dimension ICON (Bruker Corp.), ScanAsyst mode in air was used for determination. Silicon Tip on Nitride Lever SCANASYST-AIR with spring constant 0.4 N/m was used. NanoScope Analysis software was applied for data processing. The mean roughness values (R_a) represent the average of the deviations from the centre plane of the sample.

The UV-Vis spectra were measured using a Perkin Elmer Lambda 25 spectrometer in the spectral range from 225 to 400 nm. The applicable range is 190–1100 nm with bandwidth of 1 nm (fixed).

The electrical discontinuity/continuity of a doped PLLA was examined by measuring electrical sheet resistance (R_s). For determination of R_s by standard Ohm's method using KEITHLEY 487 pico-ampermeter was used. Two Au contact (50 nm thick) were sputtered on the layer surface for resistance measurement. Typical error of the measurement was ± 5%.

Thermal treatment of the polymers was accomplished in thermostat BINDER. The samples were heated for 60 min at 60 °C and then they were cooled down to the room temperature.

Electrokinetic Analysis, zeta potential determination of all samples was accomplished on SurPASS Instrument (Anton Paar GmbH, Austria) by two methods (streaming current and streaming potential) and calculated by two equations (Helmholtz-Smoluchowski, HS, and Fairbrother-Mastins, FM). Samples were studied inside the adjustable gap cell with an electrolyte of 0.001 mol dm^{-3} KCl at constant pH = 6.1 and at room temperature. Two samples of each surface were measured four times with the relative error of 5%.

2.4. Study of cytocompatibility

For cell culture experiments, pristine PLLA samples, the samples with graphene nanoplatelets (up to 10%) and GNPs doped PLLA treated with plasma discharge (3 W, 240 s) were chosen. The samples were sterilized for 1 h in ethanol (75%), air-dried, inserted into polystyrene 12-well plates (TPP, Switzerland; well diameter 20 mm) and seeded with vascular smooth muscle cells (VSMCs) derived from the rat aorta by an explantation method. VSMCs were seeded on the samples with the density of 50.000 cells/well (i.e., about 17,000 cells cm^{-2}) into 3 ml of Dulbecco's modified Eagle's Minimum Essential Medium (DMEM; Sigma, USA, Cat. No. D5648), containing 10% fetal bovine serum (FBS; Sebak GmbH, Aidenbach, Germany). Cells were cultivated at 37 °C in a humidified air atmosphere containing 5% of CO_2.

The number and the morphology of initially adhered cells were evaluated 24, 72 and 144 h after seeding. The cell proliferation activity was estimated from the increase in the cell numbers achieved on the 3rd and 6th days after seeding. PBS (phosphate buffer) was used for cell