SURFACE MODIFICATION OF POLY-L-LACTIC ACID BY GOLD SPUTTERING AND SUBSEQUENT ANNEALING

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Abstract

Self-assembly techniques attracted a lot of interest in recent years due to its ability to form number of periodic surface patterns over large areas. This process is fast, very efficient and cost effective. By modifying surface pattern we can optimize polymer for specific use, from optical devices through particle sorting to flexible electronics. Today`s main challenge is find suitable materials capable of self-assembly and study how we can influence surface pattern to achieve best results.

In our work, we used formation of ripple-like patterns by wrinkling instability of poly-L-lactic foil with gold nanostructure initiated by annealing. Resulting structures showed significant degree of morphological orientation observed by atomic force, which in optimized cases resulted in electrical anisotropy. This reorganization of conductive gold on insulating polymer into metal nanowires can be also observed by zeta-potential measurements. Due to poly-L-lactic acid being biopolymer, these samples were also tested for cytocompatibility. NIH 3T3 fibroblasts were used and selected samples showed significantly higher adhesion and proliferation in comparison with PS mock.

Keywords: Atomic force microscopy, Metal nanolayer, Self-assembly, Surface modification, Zeta potential

1. INTRODUCTION

Self-assembled structures attracted a lot of interest of researchers in recent years, as they allow formation of well defined structures over large areas in fast, cost-efficient and simple way. Goal of current research is to fine-tune their mechanical, optical, electrical, magnetic, biological and catalytic properties to suit their respective utilization [1].

Poly-L-lactic acid (PLLA) is biodegradable polymer prepared from renewable resources and have shown interesting properties for self-assembly. Samples treated with plasma [2] or sputtered with metal nanolayer [3-5] have shown formation of ripple-like structures due to wrinkling instability [6]. Such structures exhibit increased biocompatibility in comparison with pristine polymer [2] and in some cases metal forms perpendicular conductive wires over the surface [5].

Aim of this work was to present useful properties of ripple-like structures formed after annealing on PLLA with sputtered gold nanolayer.

2. EXPERIMENTAL

2.1. Materials

Biopolymer poly-*L*-lactic acid (PLLA, density 1.25 g·cm^{-3}, glass transition temperature T_g = 60°C, crystallinity 60-70 %, thickness 50 μm, Goodfellow, Ltd., UK) was used in the presented experiments.

Gold layers were deposited from Au target (99.999 %) by diode sputtering technique (BAL-TEC SCD 050 equipment, Switzerland). Typical sputtering conditions were: room temperature, sputtering times 10–300 s,

argon pressure of about 5 Pa, electrode distance of 50 mm, electric current of 20 mA. Part of the samples were annealed at 60°C for 60 min (BINDER thermostat, Germany) and then cooled to room temperature in air.

2.2. Apparatus and procedures

Surface morphology and roughness of the samples were examined by means of atomic force microscopy (AFM). The AFM images were taken on VEECO CP II setup (Veeco, USA) in a tapping mode. Si probe RTESPA-CP with the spring constant 20-80 $N \cdot m^{-1}$ was used. Roughness (R_a) represents the arithmetic average of the deviations from the centre plane of the sample. Four areas of each sample were scanned in order to get representative data.

The electrical continuity/discontinuity of the as-sputtered and annealed gold layers was inspected by determination of electrical sheet resistance (R_S). The measurement was carried out with KEITHLEY 487 pico-ampermeter by standard Ohm's method. Two Au contacts (thickness about 50 nm) were sputtered on the layer's surface for this measurement. For each point, three samples were used, each sample was measured 4 times, error was less than 5 %.

Electrokinetic analyses (determination of zeta potential) of pristine, sputtered and annealed samples were accomplished on SurPASS Instrument (Anton Paar, Austria). The samples were studied inside an adjustable gap cell in contact with electrolyte (0.001 $mol \cdot dm^{-3}$ KCl). Streaming current method was applied and zeta potential was calculated by Helmholtz-Smoluchowski equation [7]. All samples were measured four times at constant pH equal to 6.4 with the relative error of 5 %.

Cytocompatibility of the prepared samples was studied using mouse embryonic fibroblasts (NIH 3T3, generation time 20-22 h) as a model cell line. Incubation times with the tested samples in DMEM medium were following: 6 h for cell adhesion, and 24, 60 and 120 h for cell proliferation. Cells intended for analysis were fixed by 4 % formaldehyde solution in phosphate buffered saline. F-actin of the cells was stained with phalloidin-TRITC (1 $\mu g \cdot ml^{-1}$, 12 min) and cell nuclei with DAPI (0.5 $\mu g \cdot ml^{-1}$, 7 min). The cell number and morphology were studied by inverse fluorescence microscope Olympus IX-81 (Cell^R System, 150 W xenon arc burner). The cells images were captured by EM-CCD camera C9100-02 (Hamamatsu, Germany). The cell number was evaluated using ImageJ 1.43 software. For each value three samples were used and evaluated.

3. RESULTS AND DISCUSSION

Fig. 1 shows AFM scans of prepared PLLA samples. A) is as-sputtered polymer with 10 nm gold nanolayer. We observed formation of metal clusters over the surface. Similar scans were obtained for as-sputtered polymer with 5 and 40 nm gold nanolayer. B), C) and D) show significant morphological changes after annealing. We observe formation of ripple-like structures that grow with thickness of sputtered gold nanolayer. These structures also show preferred orientation over large areas (at least several centimeters), while this effect is most prominent on samples with 10 nm gold nanolayer. On these samples ridges are periodically separated by valleys.

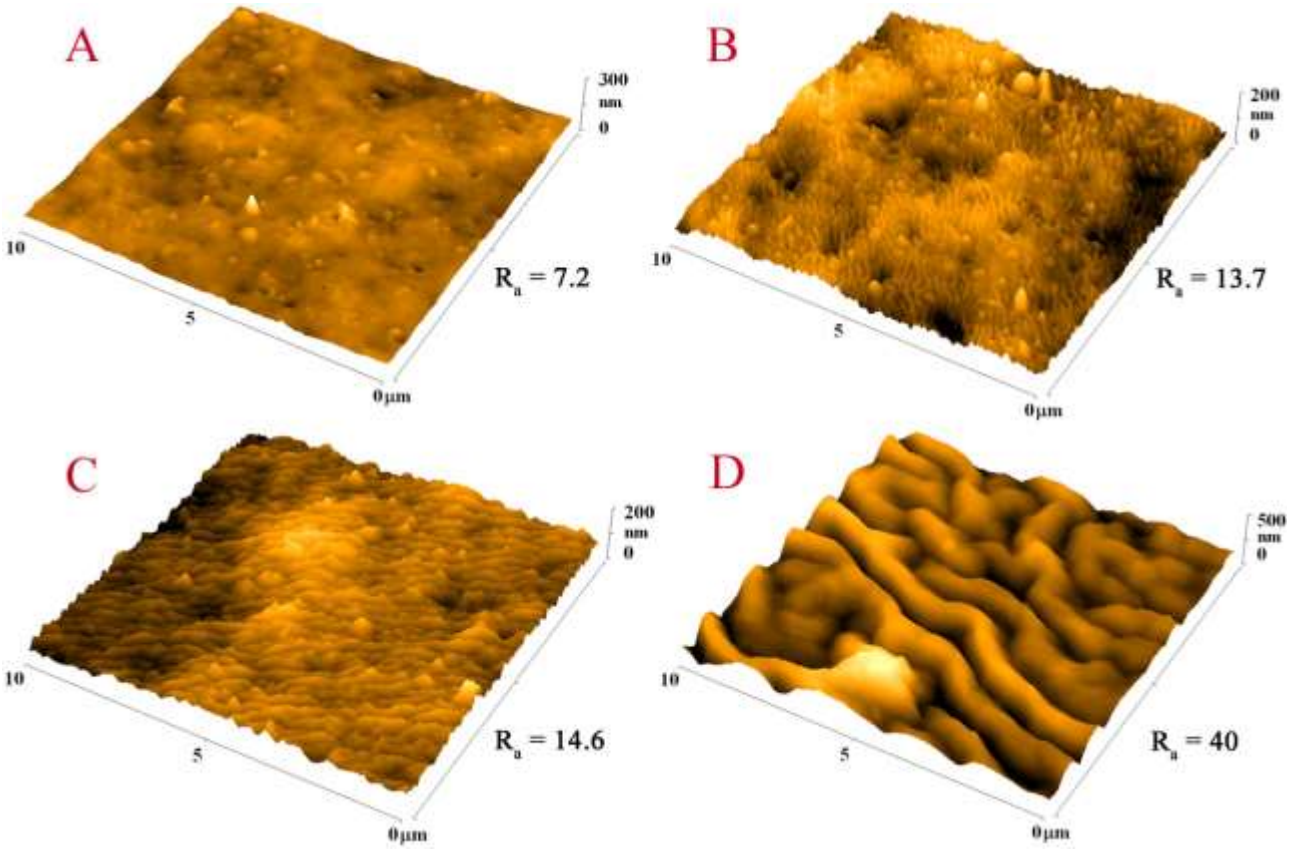

Fig. 1 AFM Scans of samples. A) As-sputtered PLLA with 10 nm Au B) Annealed PLLA with 5 nm Au C) Annealed PLLA with 10 nm D) Annealed PLLA with 40 nm

Effect of this orientation and periodic structure can be observed by sheet resistance measurement, as shown on Fig. 2. Samples on which ripples connect contacts have low sheet resistance, while samples with ripples parallel with contacts have very high sheet resistance. These results show uneven distribution of gold on annealed samples, we can safely assume surface consist of golden nanowires, that align with ripples separated by insulating polymer gaps.

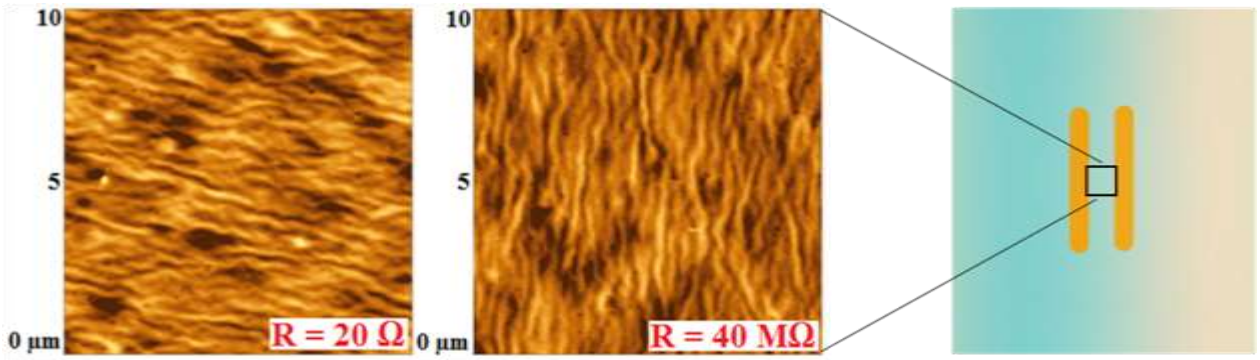

Fig. 2 AFM scans with corresponding sheet resistance based on morphological orientation of wrinkles towards contacts.

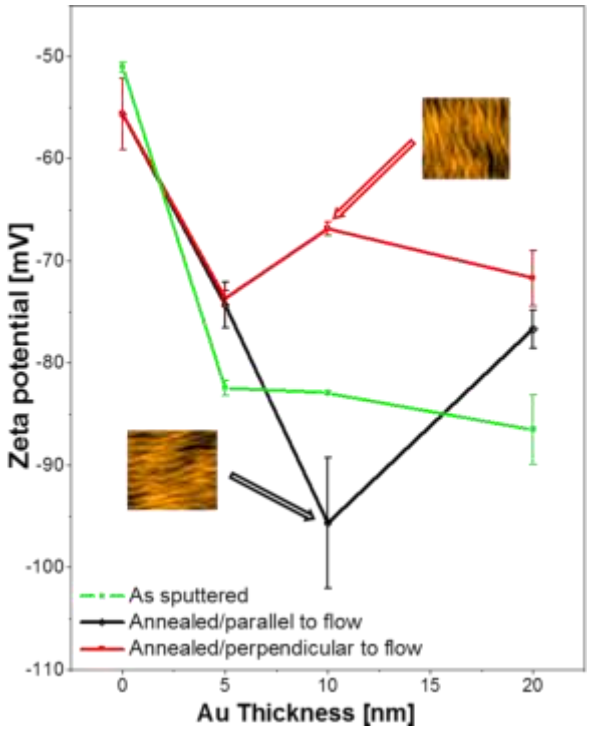

Fig. 3 Dependence of zeta potential on the Au nanolayer thickness for pristine PLLA and PLLA samples as-sputtered (filled points) and annealed (empty points). AFM scans illustrate orientation of wrinkles for given set (flow of electrolyte is from left to right).

Another effect of ripple orientation and uneven distribution of metal on surface manifest itself during zeta-potential measurement which results are shown in Fig. 3. While zeta-potential of as sputtered samples is around – 83 mV with no significant change based on gold layer thickness, annealed samples behave differently. Samples with 5 nm gold nanolayer form only small ripples (as shown in Fig. 1.) that slightly increase zeta-potential. For samples with 10 nm of gold, we observe significant dependence on ripple orientation which is in good correlation with both AFM (Fig. 1.) and sheet resistance measurement (Fig. 2.). Samples, where electrolyte flow parallel to ripples, we observe decrease in zeta-potential, in opposite situation, when flow of electrolyte is perpendicular to ripples, zeta-potential increase. This difference is result of different surface chemistry of ridges and valleys and fact, that perpendicular flow can be influenced by ridges only, while parallel flow is influenced by both ridges and valleys.

These characteristics in combination with PLLA being biopolymer showed to be beneficial for cell adhesion and proliferation. Results of bicomplatibility test are shown in Fig. 4. We can observe significantly higher cell count on both pristine and modified PLLA samples in comparison with poly-styrene (PS) mock. Pristine PLLA shows highest cell density but cells were not spread. Annealed samples show slight decrease in cell count but still higher when compared to PS mock, but more importantly samples with 3 and 10 nm showed growth of physiologically looking cells with normal cell connections.

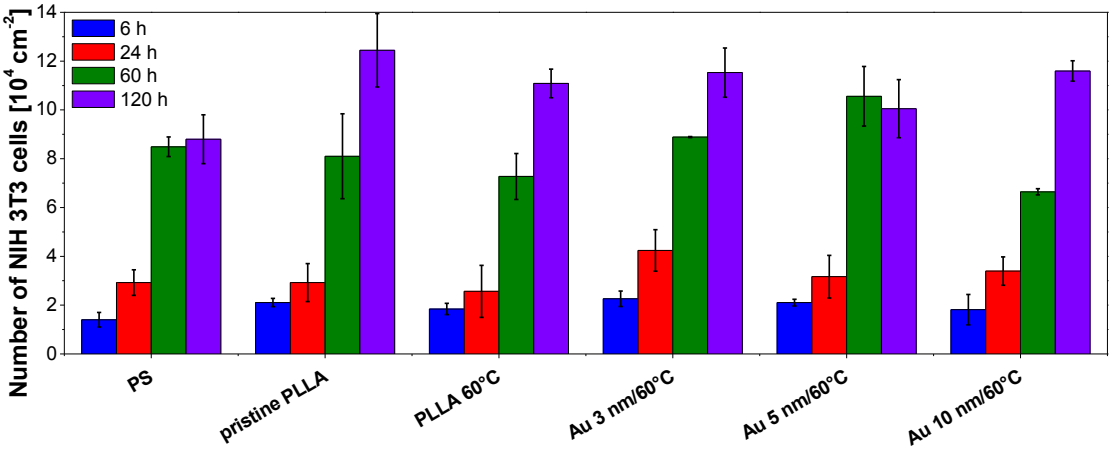

Fig. 4 The number of NIH 3T3 cells on different polymer scaffolds

4. CONCLUSION

This paper presented most important features of self assembled nanostructures formed on PLLA with sputtered gold nanolayer after annealing. We observed formation of of ripple-like structure with AFM. We determined that size of ripples I related in direct proportion to thickness of sputtered gold layer. These structures showed preferred orientation, while this effect was most prominent on samples with 10 nm gold layer. This orientation had impact on sheet resistance and zeta-potential measurement as results depended on ripple orientation towards contacts or electrolyte flow respectively.

We also tested prepared samples for biocompatibility with NIH 3T3 cells. These test showed significant increase of cell density in comparison with PS mock. While pristine PLLA showed highest cell count, it also caused growth of non-spread cells. Modifying PLLA with sputtered gold and annealing slightly decreased cell density in comparison with pristine sample, but resulted in growth of physiologically looking cells with normal cell connections.

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