Ag AND Pd NANOSTRUCTURES: FROM MATERIAL PROPERTIES TO BIOLOGICAL APPLICATIONS

Markéta KAIMLOVÁ, Markéta PIŠLOVÁ, Václav ŠVORČÍK, Jakub SIEGEL

Department of Solid State Engineering, University of Chemistry and Technology Prague, Prague, Czech Republic, EU, polivkoa@vscht.cz

Abstract

Excellent workability of polymers brings them a considerable potential to be used as matrices for metal nanostructures broadly applicable in medicine. For instance, biologically active metal/polymer composites could be effectively prepared by biocompatible polymer coating by thin metal nanolayers. Together with the fact that biological properties of such prepared composites could be improved with increasing metal surface area, we nowadays have a great opportunities for the preparation strongly effective materials. In this study, we prepared Ag and Pd nanolayers (NLs) on the surface of polyethylene naphthalate by means of cathode sputtering. Such NLs were transformed into the form of nanoislands (NIs) by low temperature post-deposition annealing. Subsequently, biological properties of commonly used NLs were compared to novel ones (NIs) with considerably higher specific surface area. Surprisingly, increase of both surface roughness and specific surface area caused by NIs formation did not support improved biological response.

Keywords: Silver, palladium, nanostructures, sputtering, annealing, biological properties

1. INTRODUCTION

In particular, long-term stability and excellent workability make polymers suitable matrices for nanostructure-based composites, especially those containing metal components. The combination of polymeric materials with metal nanostructures often brings an advantage of excellent mechanical, optical, catalytic, electrical or magnetic properties, which are significantly different unlike the bulk state of material [1]. Thus, the dominating mechanical characteristics of polymeric matrices and antimicrobial potency of metal nanostructures may significantly improve such composite properties in the specific area of use. Especially silver nanostructures, entering 3rd decade of prosperous usage in polymeric composites, exhibit excellent electrical, optical and magnetic properties, of which the most promising is undoubtedly their antibacterial effect, widely used in medicine [2]. Composite medical devices, however, must fulfil the criteria of biocompatibility and antibacterial effects. Modern nanotechnology nowadays opens up new possibilities to improve these properties and minimizes the risk of side-effect due to the massive application of these materials [3].

The improvement of biological properties can be accomplished by several methods. Modification of surface morphology and roughness has already been referred by several studies [4-6]. The alteration of these parameters might significantly improve biocompatibility of material promoting cell adhesion and proliferation [6]. To add the antibacterial efficacy to polymeric materials, one can prepare metal nanolayers on their surface. Nevertheless, one can increase the antibacterially active surface area using nanoislands as default structures. Possible way is thermally induced transformation of nanolayers into island-like, which goes hand in hand with the alteration of resulting surface morphology and roughness. Thereafter, the increase of metal specific surface area might lead to the improved antibacterial response. The formation of island-like structures by low temperature annealing of nanolayers has already been described for silver [4], and palladium [5]. Thus, the appropriately performed surface modification enables a direct control over the biological properties of composite materials.

In this work we studied the influence of the surface morphology on resulting biological properties. Metal nanolayers (common Ag and novel Pd) were sputtered on the surface of polyethylene naphthalate.

Subsequently, the samples were annealed and originally continuous layers were transformed into the form of nanoislands with considerably increased specific surface area. Surface properties of each sample were characterized by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). Evaluation of biological suitability of prepared coatings was performed by their mutual antibacterial and cytotoxicity testing.

2. EXPERIMENTAL

2.1. Materials, apparatus and procedures

Polyethylene naphthalate foil (PEN, thickness of 50 μm, Goodfellow Ltd., UK) was used as a substrate. PEN samples (Ø 2 cm) were deposited by metals on BAL-TEC SputterCoater SCD 050 device in the range of sputtering times of 10-200 s using 99.999 % pure Ag and Pd targets (Goodfellow Ltd., UK). The deposition parameters were: 20 °C, current of 15 mA and total Ar pressure of 5 Pa (99.99 % purity). Samples annealing was conducted in a Binder thermostat in air atmosphere at 250 °C for 1 h. Then the samples were cooled down and stored under laboratory conditions.

2.2. Analytical methods

Effective thickness of metal layers was determined by AFM scratch test [7] on glass substrate by AFM VEECO CP II device. The series of the samples of various metal thicknesses (sputtering times of 10-200 s) were acquired, from which 20 nm thick ones (and corresponding annealed alternatives) were chosen as representatives. Variations of the thickness did not exceed 5 %.

Compositional changes induced by the deposition and annealing process were studied by XPS. The atomic concentrations of silver Ag(3d), palladium Pd(3d), carbon C(1s) and oxygen O(1s) were measured using Omicron Nanotechnology ESCAProbeP spectrometer. The electron take-off angle was set to 0° which means typical access depth of 8-10 atomic layers [8]. The X-ray source was monochromated at 1486.7 eV (step size of 0.05 eV). The spectra were evaluated by CasaXPS software.

Surface morphology and roughness of the samples were studied on AFM VEECO CP II device working in tapping mode to minimize a potential damage of the sample surface. Bruker Antimony-doped Silicon probe CONT20A-CP attached to a flexible micro-cantilever was used near its resonant frequency of 300 kHz. The scans were measured at the line scanning rate of 0.5 Hz. Surface roughness, characterized by the mean roughness value (R_a), represents the arithmetic average of the deviation from the center plane of a sample.

2.3. Biological assays

Antibacterial potency of prepared coatings was investigated by drop plate method [9] using Gram-negative (G^-) *Escherichia coli* (DBM 3138) and Gram-positive (G^+) *Staphylococcus epidermidis* (DBM 3179). Tested and control samples (physiological solution, PS) in triplicates were immersed in 2 ml of PS, and inoculated with 1.1×10^4 of colony forming units (CFU) per 1 ml of *E. coli* and 2.2×10^4 of CFU per 1 ml of *S. epidermidis*. The samples were incubated under both static (laboratory table) and dynamic (shaking at 130 rpm) conditions at 24 °C for 3 and 24 h. Afterwards, the aliquots of 25 μl from each sample were placed on agar plates (LB for *E. coli*, PCA for *S. epidermidis*) in 10-fold repetitions. After overnight incubation, CFUs were counted by Lucia Image 4.8 software. These experiments were accomplished under sterile conditions.

Cytotoxicity was examined by WST-1 assay [10] using mouse embryonic fibroblasts (L929). The samples were sterilized by UV irradiation, inserted into 12-well plates (VWR, USA, Ø 2.14 cm), and seeded with 30,000 cells per well in triplicates in 1 ml of Minimum Essential Medium (MEM) supplemented with 10 % Fetal Bovine Serum. The cells were incubated for 24, 48 and 72 h (cultivation conditions: 37 °C, 5 % CO_2, and 95 % humidity). The culture medium was replaced with fresh phenol red free FluoroBrite DMEM (480 μl)

supplemented with 20 µl of WST-1 reagent per well. After 2 h of incubation, the medium was aliquoted into 96-microtiter plates. Then, the absorbance was measured at 450 nm (reference 630 nm) using UV-Vis spectrometer BioRad. Cells cultivated on standard tissue culture polystyrene (TCPS) served as controls.

3. RESULTS AND DISCUSSION

3.1. Preparation and surface characterization

Atomic concentrations of elements (at. %) were studied by XPS analysis (**Table 1**). Determined concentrations of C and O corresponded well to the PEN stoichiometry with mild distortion caused by hydrocarbon impurities adsorbed from air [11]. Detected concentrations of metals (Ag, Pd) were given by sputtering process. One can see that annealing of pristine PEN led to insignificant differences in its chemical composition. Compared to as-sputtered samples annealing caused a diffusion and aggregation of metals which decreased the detected concentrations of Ag and Pd in case of Ag NIs/PEN and Pd NIs/PEN, respectively. Simultaneously, element concentrations originating from the underlying polymer (C and O) were increased due to PEN substrate become partially uncovered. This phenomenon has already been studied in detail by determining the rate of surface ablation (XPS) and metal release (ICP-MS) [5]. This study uncovered that compositional changes after annealing were caused by the coalescence of metal into separate nanoislands with partial embedding of these clusters into polymer interior. Principally, the ultrathin (ones of nm) polymer overlay covered metal islands reaching almost to their tops. This phenomenon being known as “curtain effect”. Considerably higher concentration of O in Ag NIs/PEN after annealing was presumably caused by higher propensity of Ag to oxidation process.

Table 1 Atomic concentrations of C(1s), O(1s), Ag(3d) and Pd (3d) measured by XPS

Sample	Atomic concentrations of elements (at. %)			
	C	O	Ag	Pd
Pristine PEN	72.2	27.8	-	-
Annealed PEN	73.1	26.9	-	-
Ag NLs/PEN	58.4	12.0	29.6	-
Ag NIs/PEN	61.5	30.1	8.4	-
Pd NLs/PEN	73.5	1.2	-	25.3
Pd NIs/PEN	65.6	18.3	-	16.1

Because the surface morphology and roughness has a great impact on resulting biological properties of materials, AFM analysis was involved in this study. In **Figure 1**, one can see that the surfaces of pristine PEN and as-sputtered samples of both Ag and Pd NLs/PEN were mildly corrugated and similar values of R_a (**Table 2**) were measured. Compared to as-sputtered samples (see **Figure 1a**), however, noticeable alteration of the surface morphology of both metal-coated samples can be observed after the annealing (**Figure 1b**). The samples´ surface was completely rearranged during annealing process; thermal accumulation in the metal layers resulted in an alteration of the amorphous phase of PEN (T_g^{PEN} = 120 °C), which led to the significant increase of the $R_{a\ of}$ annealed samples (**Table 2**). The values of surface roughness R_a increased two orders of magnitude compared to as-sputtered samples. Isolated island-like structures (NIs) homogeneously distributed over the surface of PEN are apparent from AFM scans of both Ag and Pd/PEN samples. This phenomenon is in accordance with the results of XPS analysis (see **Table 1**), which revealed decreased values of metal concentrations in case of both Ag and Pd after annealing at the expense of the concentrations of C

and O originating from PEN substrate. Furthermore, the size and shape of metal nanoislands is effectively controllable by the thickness of metal coating preceding the annealing process [5].

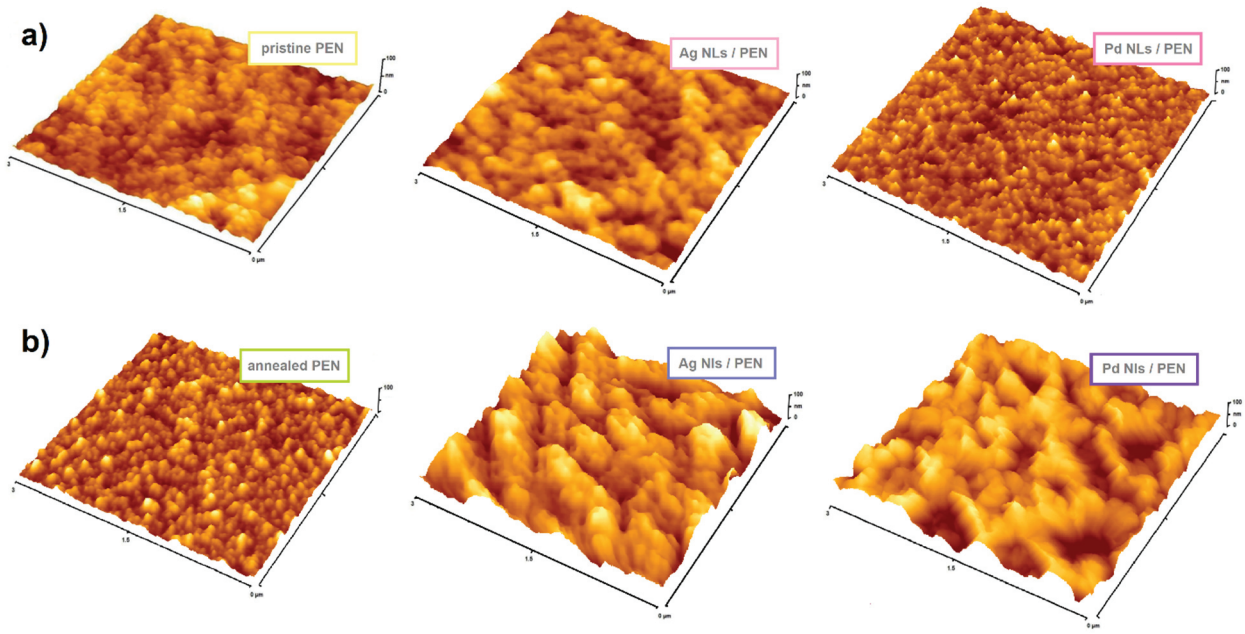

Figure 1 AFM scans of the samples a) before and b) after annealing

Table 2 Mean surface roughness (R_a) of the samples measured by AFM

Samples	Pristine PEN	Annealed PEN	Ag NLs/PEN	Ag NIs/PEN	Pd NLs/PEN	Pd NIs/PEN
R_a (nm)	4.4	4.7	4.8	151.6	4.7	148.1

3.2. Biological response

Antibacterial effects of the samples are shown in **Figures 2a,b** for *E. coli*, and *S. epidermidis*, respectively. Missing columns corresponds to no countable amount of CFU; total inhibition, respectively. Generally, pristine and annealed PEN exhibited no antibacterial effects, except an insignificant one for annealed sample after 3 h of dynamic incubation with *E. coli* (**Figure 2a**), presumably caused by a noticeable surface roughness of this substrate (see **Figure 1** AFM). Due to this fact, together with dynamic shaking at 130 rpm, bacterial colonies were mildly inconformed during their adaptation period to surrounding environment [12]. Generally, increased antibacterial effects after longer incubation time (24 h) occurred for all metal/polymer composites in case of both bacterial strains (**Figures 2a,b**) in all incubation conditions. These effects might be caused by i) increased concentrations of released metals into PS after longer incubation period (ICP-MS results were published elsewhere [5]), and ii) longer contact of bacteria with the antibacterially-active surface of the composites. When comparing the results of both metal forms, NLs and NIs ones, one can see that NLs/PEN samples exhibited similar or higher antibacterial response compared to NIs. It is in good accordance with the results of XPS (see **Table 1**) and mechanism of "curtain" effect, mentioned above. This phenomenon precluded to effectively increase the antibacterial response by the alteration of the surface morphology (increase of specific surface area of metal). When comparing the results for Ag and Pd samples, more significant antibacterial response was determined for Pd ones, in case of both types of composites. These results suggest that Pd in its diverse nanostructure forms is able to compete with commonly used Ag in its medical applications, as more antibacterial effective alternative. The comparison of the results presented in **Figures 2a** and **2b** showed generally increased antibacterial effects against *S. epidermidis* (**Figure 2b**), which was more sensitive to both metals. The most testifying are the results for both types of Pd composites, which

revealed no countable amount of CFU in all conditions. The differences in the antibacterial effects against used bacterial strains might be explained by more facile penetration of positively charged noble metals particles through the cell walls of G^+ bacteria [13].

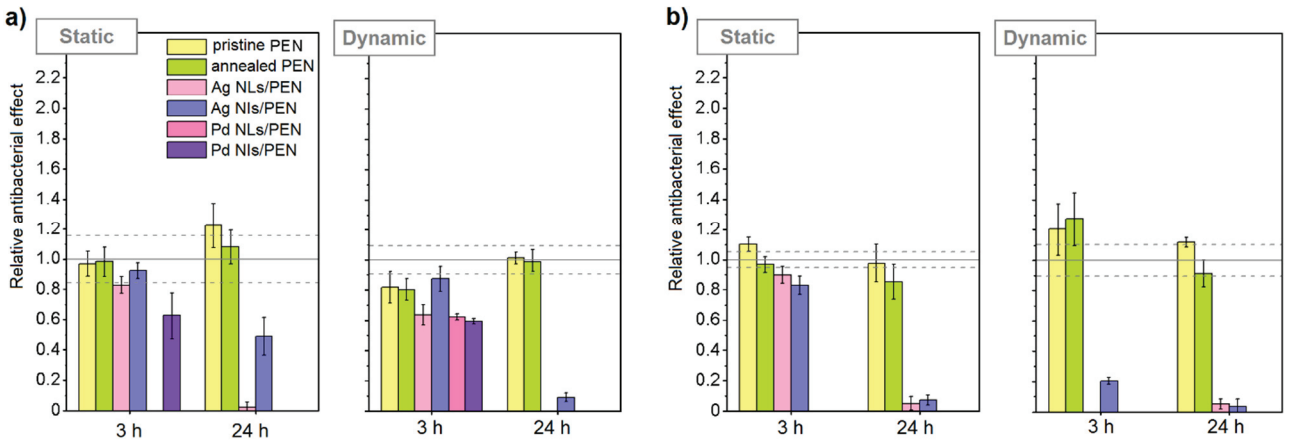

Figure 2 Relative antibacterial effect (CFU for examined sample divided by CFU for control sample) of the samples against bacterial colonies of a) *E. coli* and b) *S. epidermidis* measured in static and dynamic mode. Gray line represents a reference level (number of CFU in physiological solution) for corresponding bacterial strains together with its uncertainty (dash line).

Accessible literature [6] reports about the influence of the surface roughness on biocompatibility. Another studies [14,15], however, suggest significant cytotoxicity of metal nanostructures, especially silver ones. For these reasons, cytotoxicity assay (**Figure 3**) was performed to determine cytotoxic effects of both investigated metal nanostructure forms based on Ag and Pd.

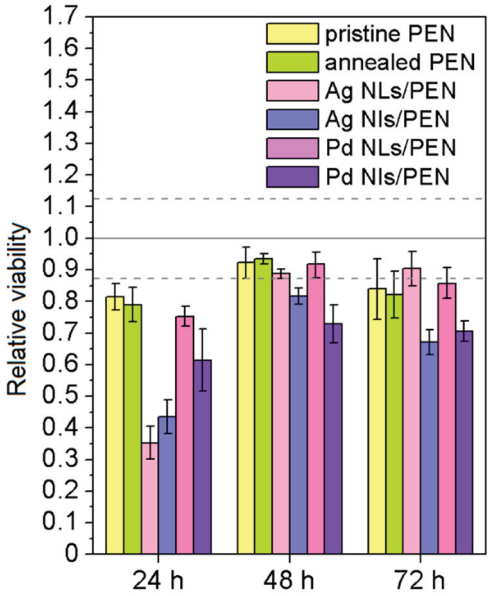

Figure 3 Relative viability (absorbance for examined sample divided by absorbance for control sample), of the samples against L929 model cell line. Gray line represents a reference level (absorbance value for TCPS) together with its uncertainty (dash line).

One can see that L929 cell viability on PEN substrate was not influenced by annealing; relative viability is comparable to control samples after longer cultivation times (48 and 72 h). Slight decrease of relative viability

observed for pristine and annealed PEN samples, alike NLs and NIs samples of both metals, after 24 h of cultivation was presumably caused by a difficult adaptation of L929 cell culture on the samples´ surface. Generally, **Figure 3** revealed low cytotoxic effects of all tested samples with minor response of NLs samples both Ag and Pd ones. Interestingly, cytotoxicity of both metals NLs samples, alike NIs ones, after longer cultivation times (48 and 72 h) was comparable; both metals demonstrated comparable cell-conformity. More significant cytotoxicity of annealed samples of both metals (NIs/PEN) compared to as-sputtered ones (NLs/PEN) was presumably caused by high sensitivity of L929 cell line to rougher surfaces [12], rather than by cytotoxic effects of metals themselves. This finding was supported the results of XPS analysis (see **Table 1**), together with "curtain" effect [5], which caused decreased amount of the metals able to evoke cytotoxicity.

4. CONCLUSION

This work presents the comprehensive study of biological effects of various kinds of metal nanostructure-based coatings of biocompatible polymer. Commonly used nanostructured Ag was confronted with Pd one. Surprisingly, it was found that the alteration of the surface morphology and roughness did not improve neither antibacterial effects of all nanostructured metal/PEN composites, due to the influence of „curtain" effect, nor biocompatibility, because of high sensitivity of L929 cell line to rougher surfaces. Both types of Ag and Pd coatings exhibited insignificant cytotoxic effects, which, together with a higher antibacterial response of Pd-coated samples, points to its potential applicability in health-care industry.

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