INFLUENCE OF THE SURFACE PROPERTIES OF TICN THIN LAYERS ON COLONIZATION BY BACTERIAL CELLS

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Abstract

Body implants are a necessary part of contemporary medicine. Biocompatibility and life-span are some of the most frequently controlled parameters of materials. One of the possible ways of ensuring these requirements is to modify the surface of the implant with a thin layer. TiCN thin films with differing compositions were deposited on AISI 316 surgical steel by the cathodic arc evaporation of pure Ti with various different gas ratios of N_2 and C_2H_2. During the deposition process, the pressure in the vacuum chamber was 1.5 Pa and the deposition temperature was 400 °C. The thickness of the layer and the adhesion of the layer to the substrate were studied from the point of view of the possible use of the samples in medical applications. The deposition parameters affect the physicochemical properties (the chemical composition, roughness and surface energy), which have a subsequent impact on the attachment of cells. Application of TiCN thin layer also increases the risk of colonization by bacterial strains. Escherichia coli was used as the model bacterial strain. Colonization of the surface that is undesirable for these applications has been observed. This work presents the physicochemical properties of the individual TiCN thin layers and also evaluates the relationship between the surface properties and colonization of the surface by bacterial cells.

Keywords: TiCN thin layers, biocompatibility, bacterial population colonization, surface roughness

1. INTRODUCTION

Adhesion of bacteria strongly correlates to surface properties. The ability of bacteria to colonize a surface is mainly related to the roughness, topography and wettability of the surface. Another parameter is the chemical composition [1].

A thin layer is usually applied to the so-called substrate to change its physical, mechanical and chemical properties. Thin layer coating is used to increase hardness, wear resistance, corrosion prevention and other unwanted chemical reactions [2]. As a result, thin films are used in electrical engineering, engineering, optics and medicine [3]. Carbon thin films have high corrosion resistance, hardness and they are chemically inert. Thanks to their biocompatibility, carbon layers are ideal for joint implant coatings. So far, DLC (Diamond Like-Carbon) layers have been used for this purpose. An alternative to DLC layers can be a carbon layer with added metal [4]. Such a metal can be titanium in the form of a TiCN (carbonitride titanium) layer, which has been evaluated as being biocompatible [5], [6].

The aim of this study is to determine the parameters that most influence the adhesion of bacteria to the TiCN thin films used in medicine [7].

2. MATERIALS AND METHODS

2.1. Description of studied samples

Seven types of thin layers of TiCN were deposit on surgical steel. Samples (S-01 to S-07) were prepared at the Central Laboratory of Applied Physics at the Bulgarian Academy of Sciences in Plovdiv. The PVD (Physical Vapor Deposition) method of Cathodic Arc Plasma Deposition was used. The deposition conditions of the individual layer types are shown in **Table 1**. A microscopic slide was used as a control during the microbiological tests. Glass was chosen as a control because it is inert and biologically inactive.

TiCN thin coatings with differing compositions are deposited by the cathodic arc evaporation of a pure titanium cathode (99.99 %) in various gas mixtures of N_2 and C_2H_2 at a constant pressure. **Table 1** shows the set ratio of C_2H_2 and N_2 in the gas flow (gas flow is measured in sccm) used for each coating. The deposition parameters, fixed for all of the coatings are: deposition temperature of 400°C and constant working pressure of 1.5 Pa. The deposition time for Sample-7 is 60 min and for all of the others it is 120 min.

Rectangles with dimensions 20 x 20 x 2 mm (AISI type 316 stainless steel) were used as a substrate. Their nominal composition is (at. %): Al - 0.08; Si - 0.9; Cr - 18.3; Mn - 1.5; Ni - 9.8; Mo - 1.2; Fe - 68.2.

The substrates are ultrasonically cleaned in acetone and ethanol for 5 minutes, followed by cleaning in a glow discharge of argon plasma and surface cleaning with metal ions. To achieve good adhesion of the coatings to the substrate, contact transition films of titanium and titanium nitride are deposited.

2.2. Measurement of physicochemical parameters of TiCN layers

Surface chemical analysis was performed on a Carl Zeiss ULTRA Plus Scanning Electron Microscope using EDX analysis. Surface roughness was evaluated using atomic force microscopy using JPK Nanowizard 3 (non-contact mode). The surface roughness (Ra) was evaluated on a scanning area of 10 x 10 µm. A wettability test was performed on the Surface Energy Evaluation System (See System). An average contact angle of ten drops was evaluated on each sample. The coating thickness is evaluated by calotest measurement. The calotest is a quick, simple and cheap method, widely used for analysing the thickness of thin coatings with thicknesses typically between 0.1 and 50 µm. The evaluation of the thickness was carried out using the integrated software on the Carl Zeiss Axio Imager optical microscope. The thin-film adhesion to the substrate was evaluated by the scratch test. This test was performed with the CETR UMT Multi-Specimen Test System (according to the EN1071-3:2005 standard). After 60 days of leaching in physiological saline, the analysis was performed and traces amounts of the Cr, Ni and Ti elements were detected in different ratios. Induced Plasma Emission Spectrometry (Perlein Elmer - Optima 2100 DV) was used.

2.3. Measurement of biological interaction with thin films

Bacteria *Escherichia coli* (strain designation: CCM No. 3954) were inoculated from a solid medium into 30 mL of soybean (Casey Digest Medium from HIEDIA) and subsequently incubated at 37 °C in an Incucell incubator from BMT Medical TechnoLogy. Based on previous experimental verification, 0.1 MCF was chosen as the most appropriate concentration of bacteria for the cultivation tests. The test with the suspension was performed at two different contact times of the bacteria with the sample surface. The samples were cleaned with cellulose moistened in acetone and dried in a Venti-cell dryer (BMT Medical Technology) at 70 °C for 30 minutes. The bacteria were exposed to the surface for one hour at 37 °C. Thereafter, the samples were shaken for 30 minutes at 250 rpm in physiological saline. The suspension was diluted to the 4^{th} order. After incubation for 48 hours at 37 °C, the number of colonies was evaluated. The whole measurement was done in triplicate. At the second attempt, the procedure was almost identical. The contact time of the bacterial suspension was extended to 24 hours and the dilution was carried out until the 6^{th} order.

3. RESULTS AND DISCUSSION

Firstly, a comparison of the physicochemical properties of the layers and the parameters of their deposition were made. In the second half of the study, the biological interaction was evaluated, and the data from microbiological tests were related to the properties of the layers.

3.1. Effect of deposition on the physicochemical parameters of TiCN layers

The chemical composition was most affected by the gas flow during deposition. A higher acetylene flow rate during deposition caused a higher percentage of carbon and oxygen in the chemical composition of the TiCN thin layer. With an increasing nitrogen flow, a higher percentage of nitrogen and titanium is formed on the deposited layer. The electric current was the only deposition parameter that had an effect on the surface roughness. A larger electric current caused a smoother surface. 3D and 2D profiles were created to evaluate the morphology of individual surfaces. Based on the definition, all of the tested surfaces are hydrophilic. According to the measurements made, none of the deposition parameters had a significant effect on surface hydrophilicity. Also, the thickness of the coated layer and the adhesion of the layer to the substrate depended on the electrical current used during deposition. Increasing the current during the coating process leads to an increase in the thickness of the deposited coatings. The higher the deposition current, the greater the adhesion of the TiCN film to the substrate. After sixty days of leaching, chromium and nickel were detected in the solution. All of the measured data are summarized in **Table 2**.

3.2. Biological interaction with thin layers

Bacterial suspension tests were performed at two different contact times of bacteria with sample surface (1 and 24 hours). After contact of the bacteria with the surface for one hour, the numbers of colonies were not significantly different. The number of colonies that colonized the surface after 24 hours is depicted in **Figure 1**. Here the differences were more pronounced. None of the chemical elements contained on the surface of a layer can be considered decisive in the colonization of bacterial populations. Surface roughness is one of the parameters that supported the formation of bacterial biofilm on the surface. The slide has a much lower surface roughness than the other samples, and the surface of the slide is noticeably less colonized than the TiCN-coated steel. Surface hydrophilicity was the most important. The less hydrophilic the surface of the sample, the better the bacteria adheres to the surface. This confirms the theory that bacteria better adhere to hydrophobic surfaces as Palmer writes in his study [8]. Surface colonization was also documented using the Carl Zeiss ULTRA Plus scanning electron microscope (**Figure 3**).

4. TABLES AND FIGURES

Table 1 Parameters of deposition of the individual layers

	Sample						
	S-01	S-02	S-03	S-04	S-05	S-06	S-07
Gas ratio C_2H_2/N_2 [sccm]	11/180	30/140	30/140	30/140	75/150	75/150	93/80
Voltage U [V]	- 40	- 40	- 60	- 40	- 40	- 40	- 40
Current I [A]	85	85	85	125	85	125	85
Deposition time t [min]	120	120	120	120	120	120	60

Table 2 Physicochemical parameters of the TiCN layers

		Sample							
		K	**S-01**	**S-02**	**S-03**	**S-04**	**S-05**	**S-06**	**S-07**
Percent chemical composition of layers [At. %]	**C**	-	9.0	32.8	30.4	23.2	47.0	38.3	53.1
	N	-	41.2	30.7	31.7	34.6	22.1	26.3	12.7
	O	-	2.5	6.9	5.4	3.4	10.4	7.0	20.2
	Ti	-	47.3	29.5	32.5	38.8	20.6	28.4	13.9
	Total		100.0	100.0	100.0	100.0	100.0	100.0	100.0
Contact angel [°]	**θ**	33.8	47.8	61.4	58.5	68.9	56.2	51.55	57.52
Layer thickness [nm]	**t**	-	664	1020	734	1534	1265	2383	1371
Scratch test [N]	**L_{C1}**	-	7.5	7.5	7.5	8.5	8.1	8.5	7.8
	L_{C3}	-	9.6	9.1	9.5	10.8	10.0	10.7	10.0
Amount of leachate [mg/l]	**Cr**	-	0.0013	0.0029	0.0019	0.6551	0.0135	0.0211	0.0038
	Ni	-	0.0004	0.0045	0.0425	0.3420	0.0435	0.0348	0.0107
	TOC	-	2.2	4.1	2.7	2.5	2.8	2.4	14.1
Surface roughness [nm]	**Ra**	4.3	99.5	151.9	104.1	73.3	141.3	63.5	118.6

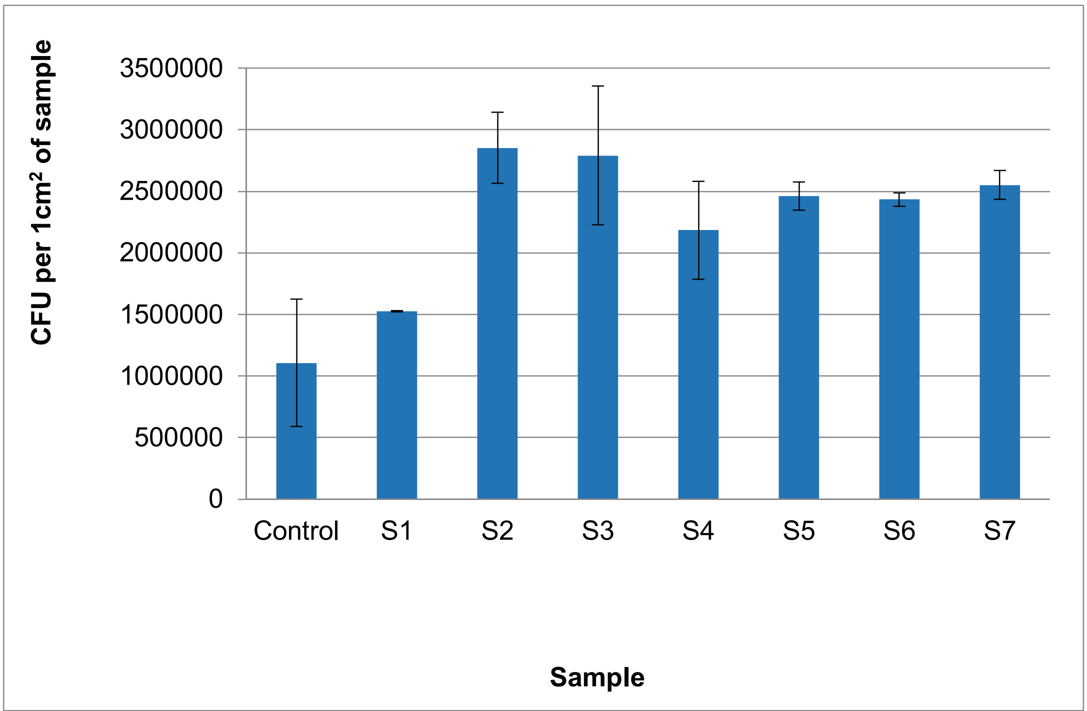
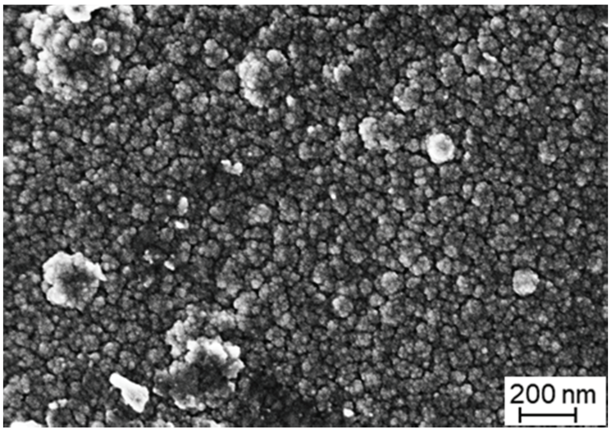
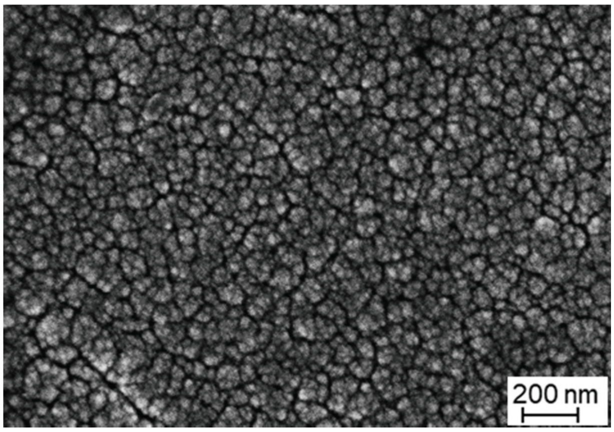


Figure 1 Colonization of the surfaces by bacterial cells after 24 hours





Sample surface (S2) Sample surface (S4)

Figure 2 The layers are applied at the same gas ratio (30/140). The increase in current from 85 A to 125 A (sample 4) results in a refinement of the structure and a reduction in biofilm formation

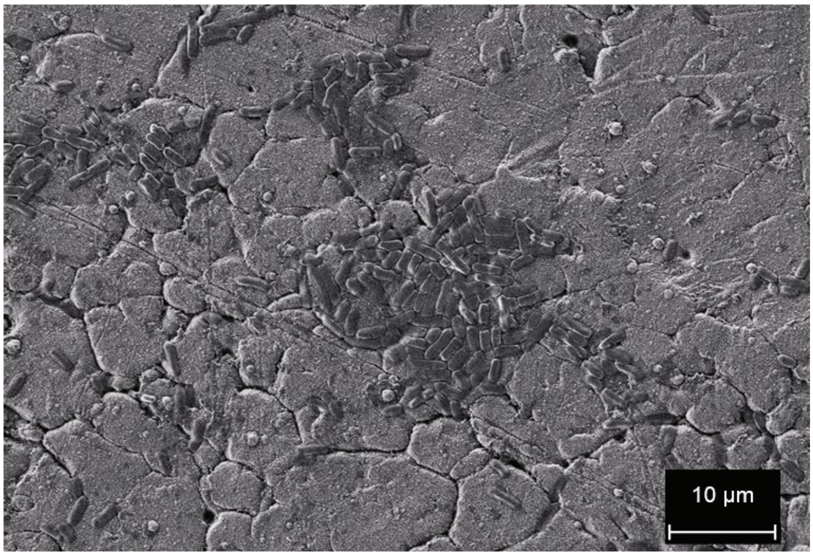


Figure 3 SEM imagine. Colonization of the surfaces by bacterial cells after 24 hours. Well-visible structure of the surface

For samples 1, 2, 5 and 7, a constant current and voltage (- 40 V and 85 A) were used. **Figure 1** shows that the change in the C_2H_2 / N_2 (11/180) gas ratio leads to differences in biofilm formation. The best results were obtained for sample 1 compared to the other samples. An increase in the gas ratio of samples 1, 2, 5 and 8 leads to an increase in surface roughness (see **Table 2**).

On the contrary, for samples 2, 3 and 4, the same ratio of gases was used. The current and voltage were changed. The voltage was increased from -40 A to -60 A. This change led to a slight decrease in the formation of biofilm on the surface (by 2.2 % compared to sample 2). Increasing the current (from 85 A to 125 A) on the sample surface decreased the creation of biofilm by 23.4 % compared to sample 2. Increasing the voltage from - 40 to - 60 V (sample 3) and the current from 85 to 125 A (sample 4) led to a refinement of the structure of the coatings, the measured surface roughness of samples 3 and 4 was lower than sample 2.

For samples 5 and 6, a constant gas ratio of 75/150 sccm and change of current from 85 A to 125 A had no effect on the formation of biofilm on the surface of the studied layers. Increasing the current from 85 to 125 A (sample 6) led to a refinement of the coating structure, i.e. a reduction of surface roughness (**Table 2**, sample 5 and 6).

5. CONCLUSION

Seven types of thin TiCN layers were deposited on AISI 316 surgical steel. The most striking effect on biofilm formation in terms of the physicochemical parameters was the hydrophobicity of the surface. The more hydrophobic layers were more colonized by bacteria. The creation of a biofilm increased by 258 % on sample 2 (the most hydrophobic) compared to the control. A higher surface roughness also had a positive effect on biofilm formation, i.e. a rougher surface led to a better adhesion of bacteria and faster biofilm formation. For the samples with the TiCN layers, the content of the individual elements on the surface had a minimal effect on biofilm formation. The aim of this work was to reveal the dependence between the properties that reduce the adhesion of bacteria cells to the surfaces and the deposition parameters of the layer. Based on the performed tests, it was concluded that increasing the current during the coating process leads to a reduction of surface roughness (see **Table 2**, samples 4 and 6) and thereby an decrease in bacterial biofilm formation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance provided by the Research Infrastructures NanoEnviCz (Project No. LM2015073) supported by the Ministry of Education, Youth and Sports of the Czech Republic and the project Pro-NanoEnviCz (Reg. No. CZ.02.1.01/0.0/0.0/16_013/0001821) supported by the Ministry of Education , Youth and Sports of the Czech Republic and the European Union - European Structural and Investments Funds in the frame of Operational Programme Research Development and Education.

The results of this project LO1201 were obtained through the financial support of the Ministry of Education, Youth and Sports in the framework of the targeted support of the "National Programme for Sustainability I" and the OPR&DI project Centre for Nanomaterials, Advanced Technologies and Innovation CZ.1.05/2.1.00/01.0005.

The work of A. Coufalová was supported by the Ministry of Education of the Czech Republic through the SGS project no. 21176/115 of the Technical University of Liberec.

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