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STUDY OF THE MECHANICAL AND ANTIBACTERIAL PROPERTIES OF SURFACE MODIFIED STEEL FOR MEDICAL APPLICATIONS

BADANIE WŁAŚCIWOŚCI MECHANICZNYCH I ANTYBAKTERYJNYCH MODYFIKOWANEJ POWIERZCHNIOWO STALI DO ZASTOSOWAŃ MEDYCZNYCH

Key words: titanium nitride, stainless steel, biomaterials, antibacterial properties, PVD.

Abstract: Various types of metal implants, both in Poland and worldwide, are mainly manufactured from stainless steel due to their biocompatibility, strength, and relatively low price. However, any such procedure involves the risk of peri-implant infection, stimulated, among other things, by the formation of a bacterial biofilm on the surface of the implant. In this paper, several methods of modifying the surface of steel for medical applications were proposed, such as mechanical polishing, electropolishing, sandblasting, and the application of a thin surface layer. This was followed by a series of physicochemical and biological tests. The results indicate that the titanium nitride coating improved corrosion resistance and reduced bacterial adhesion on the surface. No significant improvement in abrasion was observed, and the adhesion of the coating closely depended on the method of preparation.

Słowa kluczowe: azotek tytanu, stal nierdzewna, biomateriały, własności antybakteryjne, PVD.

Streszczenie: Implanty metalowe, zarówno w Polsce, jak i na świecie, produkowane są głównie ze stali nierdzewnej ze względu na jej biokompatybilność, wytrzymałość i stosunkowo niską cenę. Jednak każdy tego rodzaju zabieg wiąże się z ryzykiem powstania zakażenia okołowszczepowego, stymulowanego m.in. powstawaniem biofilmu bakteryjnego na powierzchni implantu. W pracy zaproponowano kilka metod modyfikacji powierzchni stali do zastosowań medycznych, takich jak polerowanie mechaniczne, elektropolerowanie, piaskowanie oraz nałożenie cienkiej warstwy powierzchniowej. Następnie przeprowadzono szereg badań fizykochemicznych i biologicznych. Wyniki wskazują, że powłoka azotku tytanu poprawiła odporność na korozję oraz ograniczyła adhezję bakterii na powierzchni. Nie zaobserwowano znaczącej poprawy ścieralności, a adhezja powłoki ściśle zależała od metody jej przygotowania.

INTRODUCTION

Austenitic 316LVM stainless steel is widely used as a biomaterial because it combines good mechanical properties with decent biocompatibility **[L. 1]**. Furthermore, its low cost and machinability compared to other metallic implant materials make 316LVM steel a good alternative for medical devices. Unfortunately, with this alloy, there is a risk of ion release **[L. 2]** or the formation of a peri-implant

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infection. Stainless steel for medical applications is produced by smelting under a vacuum, which generates the high degree of purity required for implants. It has excellent resistance to tissue and physiological fluids, intergranular and general corrosion **[L. 3]**, but it is mainly used for short-term implants **[L. 4]**. Depending on the application and desired effect, different methods are used to modify the surface of the steel. Sandblasting of 316LVM stainless steel results in strong plastic deformation and an increased microhardness to a depth of about 200 μm. Previous studies have shown the possibility of significantly improving the mechanical properties of surfaces without deteriorating the magnetic response of austenitic stainless steel using the sandblasting technique; therefore, it can be considered a promising surface modification for use in medical applications **[L. 5]**. However, the high surface roughness affects the amount of protein adhesion, thrombogenicity, and tissue response, which means that the nature of the metal surface is crucial for blood compatibility. A smooth surface can help prevent platelet activation and aggregation, which is considered one of the elements of the thrombotic process **[L. 6**, **7]**. Low roughness is achieved by mechanical and electrochemical polishing. Once an implant is inserted into the body, a biofilm can form on its surface. A biofilm is a form of aggregation of bacteria, fungi, and other microscopic organisms in the form of thin deposits that form on various surfaces in contact with, for example, body fluids. The natural bacterial flora is responsible for the formation of a biofilm. Its presence can lead to atrophy of the surrounding tissue, inflammation, and the need for implant replacement or removal. Currently, infection prevention focuses on putting patients on antibiotic therapy, but nonpharmacological methods of preventing biofilm formation remain under search. One of them is the modification of the material surface, as well as the application of functional coatings **[L. 8**, **9]**.

Over the past few years, multiple attempts have been made to apply coatings to 316LVM steel, such as Diamond Like Carbon (DLC) coatings and their modifications **[L. 10**, **11]**, hydroxyapatite **[L. 12**, **13]**, titanium dioxide **[L. 14**, **15]** and titanium nitride **[L. 11**, **16**, **17]**. Titanium nitride TiN is a versatile material with a nitrogen content ranging from 37.5% to 50%. At present, it is mainly used as a coating material to extend the life of cutting tools. The combination of high hardness with good ductility and sufficient corrosion resistance in physiological fluids has made titanium nitride coatings the number one choice for wear-related medical applications, such as orthopaedic implants **[L. 18]**. TiN films are often used to increase the wear resistance of two popular metallic materials: corrosion-resistant austenitic 316L stainless steel and titanium alloy Ti6Al4V. Numerous examples of successful surface modification of these materials with TiN films have been reported in the literature. In wear tests conducted under dry sliding conditions in a ball-on-disc configuration, where the counterexample was a WC-Co ball, the wear resistance of the TiN-modified 316 L steel improved by approximately 6.5 times compared to the uncoated material **[L. 19]**.

Although TiN coatings are considered a reliable anti-wear solution, recent studies **[L. 20]** show that the anti-wear capacity of TiN-coated metallic materials can be limited by various factors. The results indicate that the mismatch in elastic modulus between the coating and the substrate is one of the key factors determining the service life of TiN-coated alloys. Even though TiN coatings are used in medical applications to enhance the tribological performance of metallic materials, numerous examples of the adverse effects of titanium nitride coatings on the wear performance of implant alloys have been reported. Flaking and cracking of the TiN coating deposited on the head of a hip implant during the friction simulator operation were reported in **[L. 21]**.

From the literature review, it can be concluded that the wear performance of TiN-coated metal alloys strongly depends on both the substrate material and the friction conditions. Implants for different applications, even from the same material, are produced differently and characterised by different surface preparation. Therefore, the purpose of this study was to experimentally verify the effects of three selected surface modifications of 316LVM steel (sandblasting, polishing, and electropolishing) on the mechanical and bacteriostatic properties of the TiN coating.

MATERIALS AND METHODS

The te st material was 316LVM steel, whose chemical composition meets ASTM F138 standard (**Table 1**). The form of the samples was discs with a diameter of d = 14 mm and a thickness of g = 3 mm. After tumbling, the material's surface was

Table 1. Chemical composition of 316LVM steel, % mass, Fe balance
Tabela 1. Skład chemiczny stali 316LVM, % masy, reszta Fe

C	Si	Mn	P	S	Cr	Mo	Ni
0.030 max.	1.0 max.	2.0 max.	0.025 max.	0.01 max.	17.0 ÷ 19.0	2.25 ÷3.0	13.0 ÷ 15.0

prepared by sandblasting, mechanical polishing, and electropolishing. Then they all were cleaned in 98% ethanol in an ultrasonic cleaner for 15 min.

The surface was sandblasted with a Micra 2 Dentalfarm sandblaster using a 90–150 µm grit glass microspheres abrasive for 2 minutes at 3 bars. Mechanical polishing was carried out on an automatic Struers sander-polisher, using 120–4000 grit papers and silica suspension for final polishing. Electrochemical polishing was carried out in a bath based on sulfuric and phosphoric acid.

The titanium nitride (TiN) layer was deposited using the Moorfield nanoPVD-S10A device. It is a magnetron sputtering system for sputtering metals or insulating materials such as oxides and nitrides. TiN (titanium nitride) target was used. The process was carried out in an argon atmosphere. The gas flow rate was 20 sccm, and the generator power was 60 W. The duration of the process was 60 minutes. To ensure uniform coating thickness, the table with the samples attached rotated at a constant speed of 3 rotations per minute. **Table 2** contains a description of the numbering of samples, 3 to 5 samples of each variant were used for each test.

Table 2. Sample labels
Tabela 2. Oznaczenia próbek

1	sandblasted
1a	sandblasted + TiN
2	polished
2a	polished + TiN
3	electropolished
3a	electropolished + TiN

SURFACE COATING ADHESION TEST

Adhesion testing of the coating to the substrate was carried out by scratch test using Micro-Combi_ tester, CSM (Anton-Paar). The tests were carried out according to PN-EN ISO 20502:2016-05, which consisted of scratching the surface using a Rockwell diamond cone.

Tribological wear tests

A ball-on-disc method using a stainless-steel ball was used to test abrasion resistance. The diameter of the ball was 6 mm, the linear velocity was 0.5cm/s, which is in the range of sliding velocities is the typical for hip joints (0–50 mm/s) **[L. 23]**, and the load was 5N **[L. 23]**.

Wettability

Wetting angle measurements were performed using the sitting drop method. Measurements were made with 1 µl of distilled water at room temperature using Surftens Universal goniometer, OEG, and Surftens 4.5 software to analyse the recorded droplet image. The time per measurement was 60 s with a sampling rate of 1Hz.

Potentiodynamic test

Potentiodynamic tests were performed to check the resistance of the test material to pitting corrosion. The tests were carried out according to ASTM F2129/ PN-EN ISO 10993-15. The test stand included an AutoLab potentiostat with Nova 2.0 software, an SCE KP-113/ Ag/ AgCl 3M KCl calomel electrode, a platinum auxiliary electrode, and an anode, which was the test specimen. Measurements were carried out in Phosphate Buffered Saline (PBS) solution, simulating the environment of the human body. The opening potential Eocp was measured for 15 min, and then the polarisation curves from $E_{start} = E_{ocp} - 100$mV at a potential change rate of 3 mV/s. When the anodic current density reached 1 mA/cm^2 or the potential reached 4 V, the polarisation direction changed. The corrosion potential E_{corr}, corrosion current density i_{corr}, and polarisation resistance R_p were measured.

Optical profilometry

Surface roughness testing was performed using an optical profilometer 3D Surface Metrology Microscope Leica DCM8. The surface scans were processed in Leica Map software, resulting in average Sa (the extension of Ra – arithmetical mean height of a line to a surface) values and 3D surface visualisations at a magnification of 20x.

Bacterial adhesion

Bacterial adhesion studies were performed using the reference *Escherichia Coli* (ATCC 25923). Before testing, the bacterial inoculate was incubated for 18 h at 37°C in a sterile 15 cm^3 Tryptic Soy Broth (TSB). The test samples were then placed in 24-well sterile plates. One ml of bacterial suspension (~$1 \cdot 10^8$ CFU/mL) was added to the test samples in a culture plate and incubated at 37°C for 4 h with shaking at 80 rpm. The samples were twice gently washed twice with sterile water. The samples were moved to a new sterile plate and intensively washed with 1 ml of 0.25% trypsin for 30 s. Then 100 µL of solution were collected and mixed with 0.9% NaCl to obtain the concentrations of 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000. 100 µL of the prepared solutions were spread on agar plates. The agar plates were incubated at 37°C for 18 h. The live bacterial colonies were counted on the agar plates. The results are presented as average results from the three independent samples.

RESULTS AND DISCUSSION

Surface coating adhesion test

The test was conducted for each of the variants with a coating to assess its adhesion to the substrate. The results are shown in **Table 3** and **Figures**

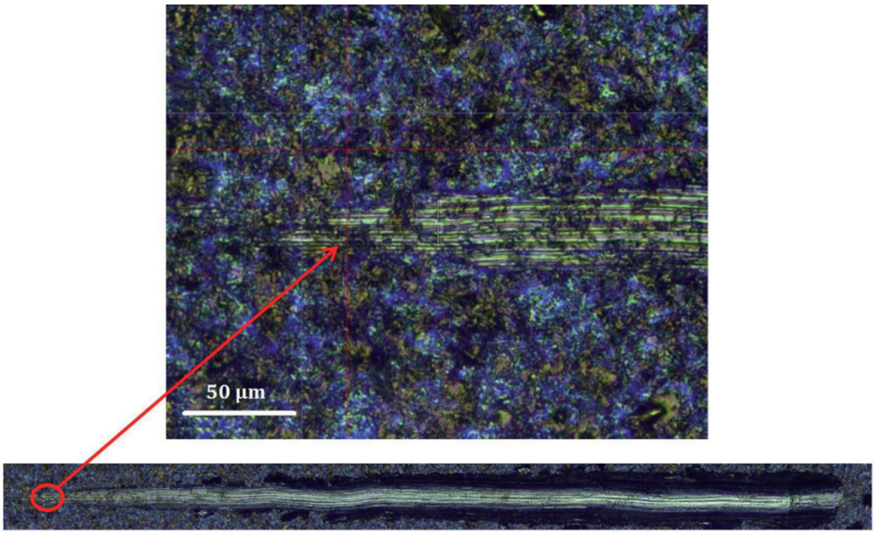


Fig. 1. Result of the scratch test for 1b, sandblasted with TiN layer
Rys. 1. Wynik próby zarysowania dla 1b, piaskowanej z warstwą TiN

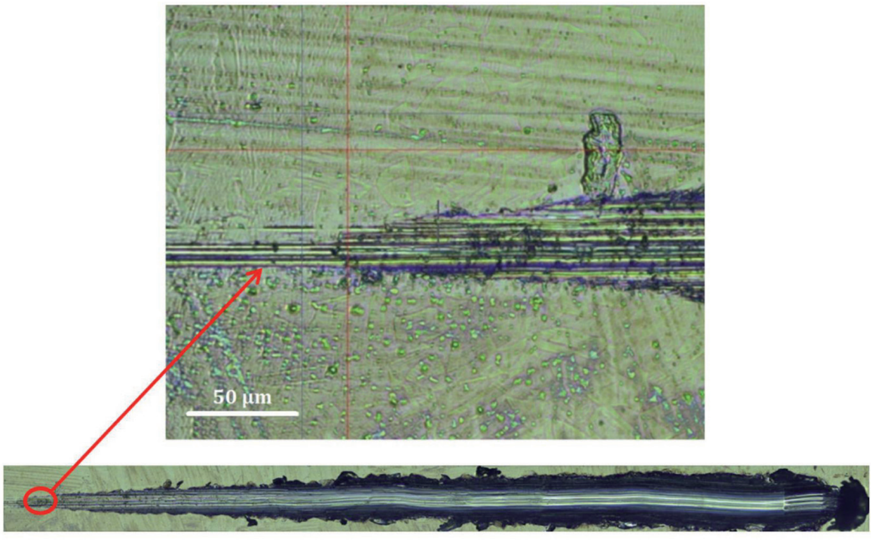


Fig. 2. Result of the scratch test for 2b, polished with TiN layer
Rys. 2. Wynik testu zarysowania dla 2b, polerowanego z warstwą TiN

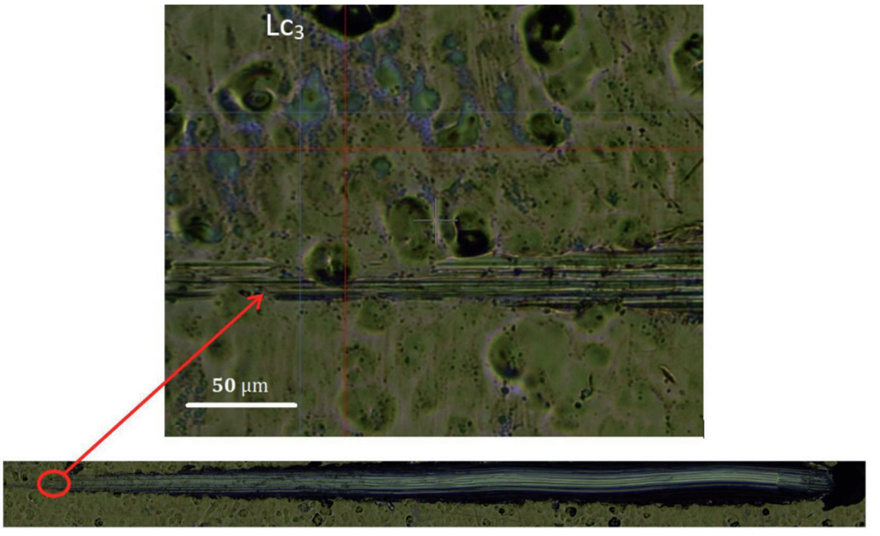


Fig. 3. Result of the scratch test for 3b, electropolished with TiN layer
Rys. 3. Wynik testu zarysowania dla 3b, elektropolerowanego warstwą TiN

Table 3. Results of adhesion tests
Tabela 3. Wyniki badań adhezji

Complete break Lc_3 [N]		
1b	sandblasted + TiN	1.20 ± 0.04
2b	polished + TiN	0.40 ± 0.03
3b	electropolished + TiN	0.19 ± 0.03

1–3. The different colors of the obtained films also confirm the effect of surface preparation on coating properties. Complete break Lc_3 is the force required to break the continuity of the coating. In each sample, continuous plastic perforation of the coating was observed. The best adhesion of the TiN coating to the substrate was observed for the sandblasted surface and the least adhesion to the electropolished surface. Despite the lower roughness, sample 2b (mechanically polished) had twice the breaking force Lc_3 of 3b (electropolished).

Tribological wear tests

The average values of the coefficient of friction are in **Table 4**, and graphs are shown in **Figure 4**. Performing the test with a steel counter ball and under 5N pressure, only the sandblasted surface shows an increase in the friction coefficient. For the other variants, the application of TiN coating did not affect the resistance to abrasion of the substrate.

The test duration for the uncoated samples was 100 seconds, and for the coated samples, it was interrupted at about 50 seconds due to the coating rubbing off. The coating was so thin that it rubbed off almost immediately after the test began. Comparing the values obtained with a similar coating **[L. 11]**, the authors obtained a friction coefficient of 0.49 for a substrate that was not subjected to any modification. Thus, it can be concluded that for the sandblasted substrate, the abrasion resistance improved, while for the polished substrate, it deteriorated.

Table 4. The average values of the coefficient of friction μ
Tabela 4. Średnie wartości współczynnika tarcia μ

1a	0.62
1b	0.65
2a	0.44
2b	0.45
3a	0.50
3b	0.51

Wettability

The results of the wetting angle measurements are shown in **Figure 5**. shows the images of the droplets placed on the investigated samples. It was observed that for each of the selected surface treatments, the TiN coating increased the wetting angle. However, all measured values were below 90°, so both the substrate and the coating were hydrophilic.

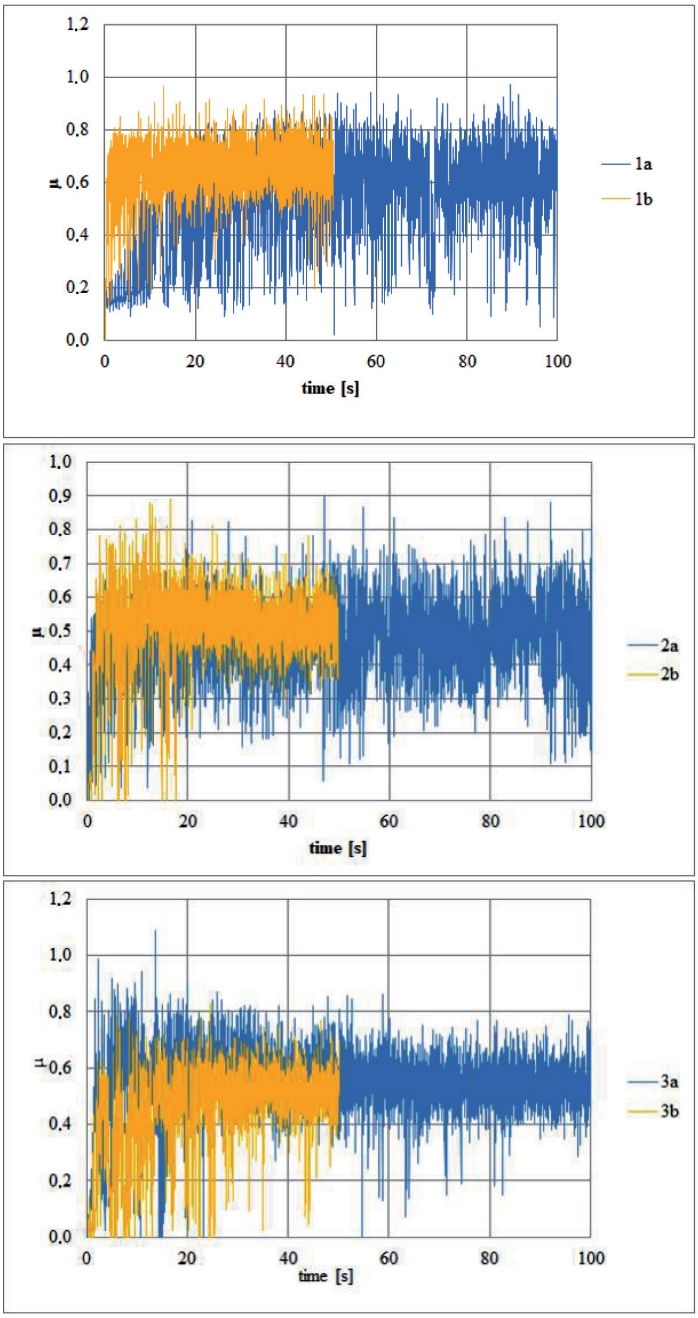


Fig. 4. Results of tribological tests – without and with TiN layer
Rys. 4. Wyniki badań tribologicznych – bez i z warstwą TiN

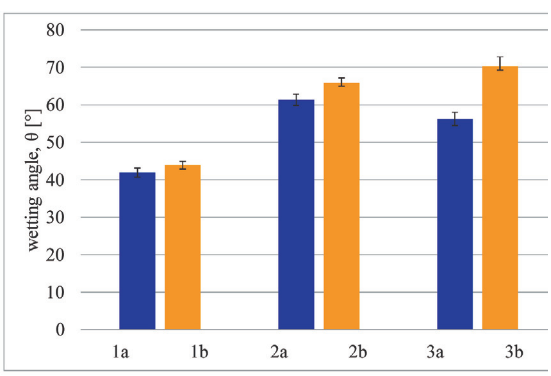


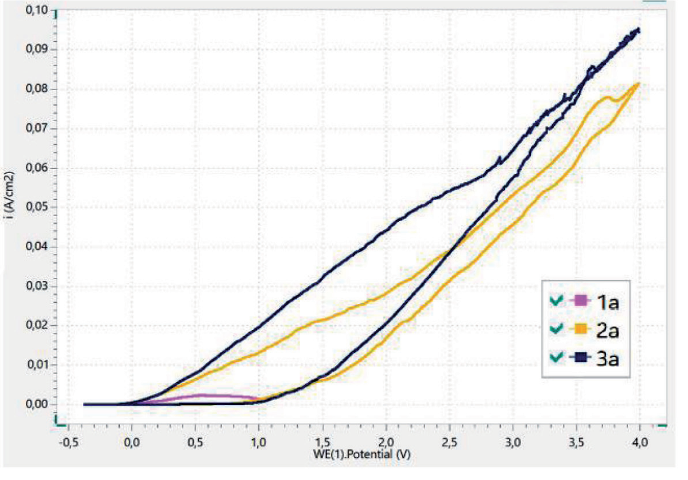
Fig. 5. The results of the wetting angle measurements
Rys. 5. Wyniki pomiaru kąta zwilżania

Potentiodynamic test

The results of corrosion resistance are shown in **Table 5**, and the recorded anodic polarisation curves are shown in **Figure 6** and **8**. The TiN coating affected the corrosion resistance of the test material. In the case of sandblasting and polishing, it influenced a decrease in E_{corr} and i_{corr}, and an increase in polarisation resistance R_p. A hysteresis loop was also created. A similar effect on R_p and i_{corr} was observed for the electropolished variant, while E_{corr} was comparable in this case. In conclusion, the TiN coating improved the corrosion resistance of the substrate in each case.

Table 5 Results of corrosion resistance
Tabela 5 Wyniki badań odporności na korozję

Surface modification	E_{corr} [mV]	R_p [kΩ·cm^2]	i_{corr} [µA/cm^2]
1a	-284 ± 12	6.89 ± 0.39	3.78 ± 0.28
1b	-341 ± 19	9.38 ± 0.36	2.78 ± 0.20
2a	-194 ± 16	5.99 ± 0.27	4.35 ± 0.39
2b	-275 ± 13	17.59 ± 0.51	1.48 ± 0.11
3a	-322 ± 12	9.70 ± 0.42	2.69 ± 0.24
3b	-313 ± 12	16.20 ± 0.76	1.61 ± 0.15



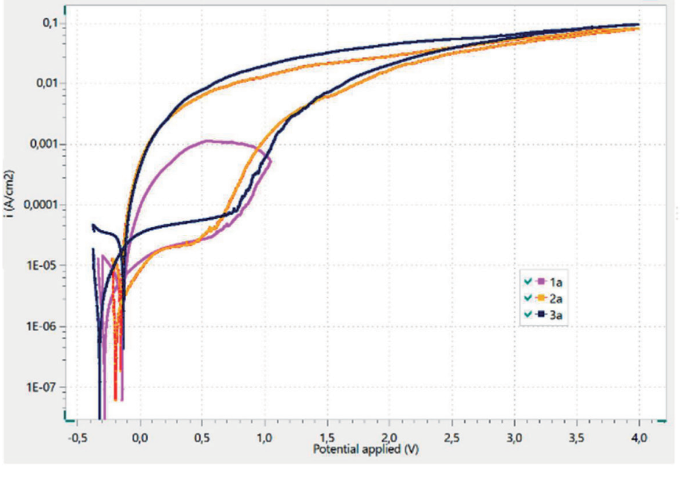


Fig. 6. Anodic polarisation curves for 316LVM steel without TiN layer
Rys. 6. Krzywe polaryzacji anodowej dla stali 316LVM bez powłoki TiN

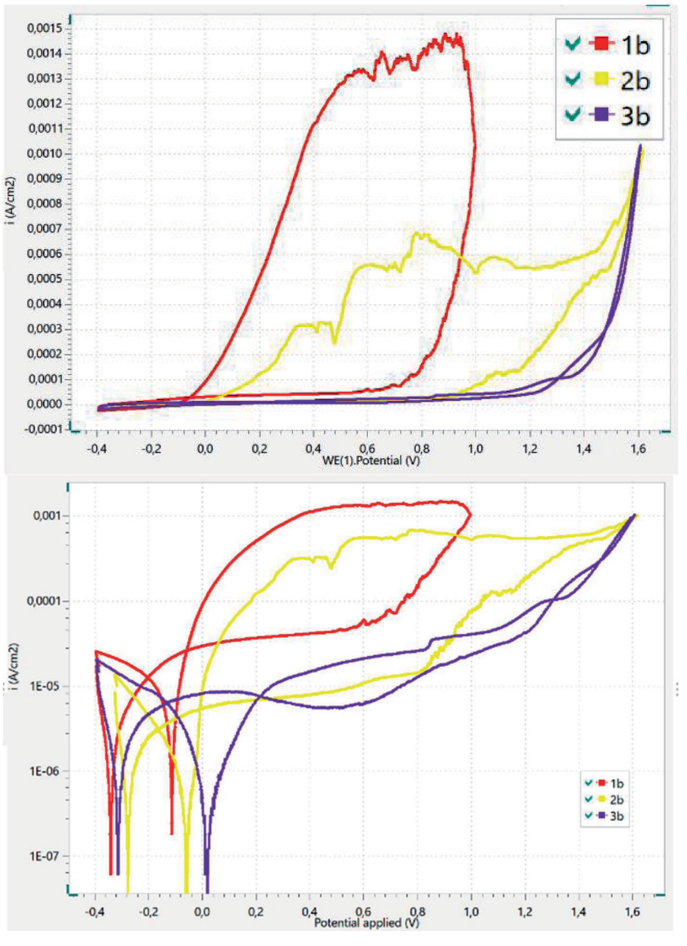


Fig. 7. Anodic polarisation curves for 316LVM steel with TiN layer
Rys. 7. Krzywe polaryzacji anodowej dla stali 316LVM po nałożeniu warstwy TiN

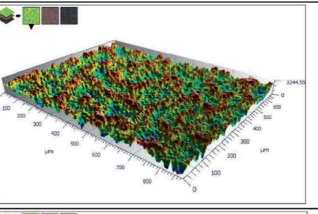
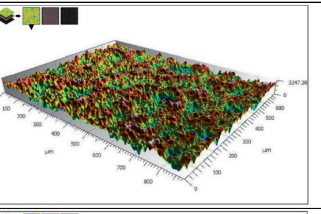
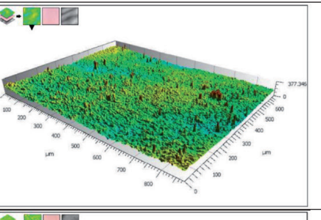
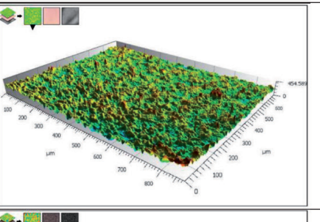
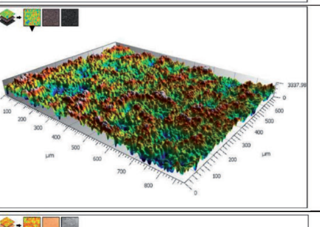
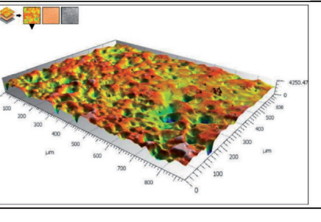
Optical profilometry

The results of surface roughness measurements indicate that the roughness of the TiN coating strictly depends on the surface preparation method. Thus, the coating slightly decreased Sa for sandblasting, and for polishing, it slightly increased it. In contrast, a significant decrease in the roughness of about 30% can be observed for electropolishing. In this case, one can also see the biggest difference in the 3D visualisation – the coating has largely filled the irregular surface after electropolishing.

Bacterial adhesion

Bacterial colonies were counted, and the results are presented in **Figure 8**. The antibacterial effect of the TiN layer was observed in the sandblasted and electropolished variant (1 + TiN, 3 + TiN). The TiN coating has improved antibacterial properties for the sandblasted and electropolished variants. For the polished surface, neither deterioration nor improvement of the adhesion of *E. Coli* bacteria was observed, while their numbers were comparable with those of the other coated variants. Procedures for assessing microbial colonisation according to ISO standards are rarely found in scientific papers, and such tests are performed when testing materials or products intended for marketing. To date, the properties of Si-DLC films produced by DC-RF multifocal magnetron sputtering can only be regarded as preliminary testing **[L. 24]**. It was shown that the introduction of a dopant into DLC coatings increases the number of E. coli cells that adhered to the tested surfaces, but microbial colonisation remains at the level of the substrate material. On the contrary, the coating significantly improved mechanical properties **[L. 10]**. A correlation was observed between surface roughness and the amount of E. coli that adheres to the surface.

Table 6. Summary of visualisation and average surface roughness (Sa) values for all variants
Tabela 6. Zestawienie wartości wizualizacji i średniej chropowatości powierzchni (Sa) wszystkich wariantów

	3D visualisation	Sa
1a sandblasted		**434 nm**
1b sandblasted + TiN		**429 nm**
2a polished		**19 nm**
2b polished + TiN		**33 nm**
3a electropolished		**489 nm**
3b electropolished + TiN		**349 nm**

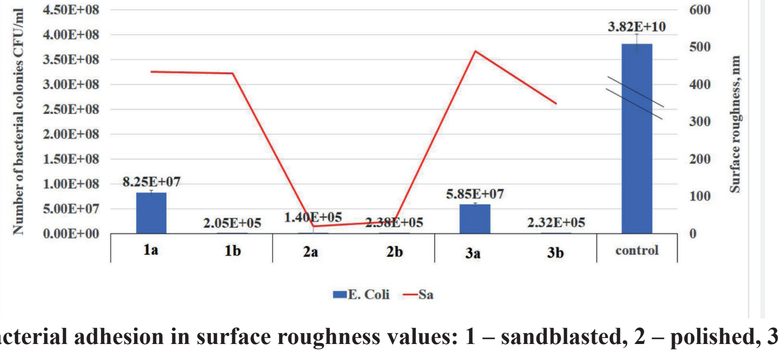


Fig. 8. Results of bacterial adhesion in surface roughness values: 1 – sandblasted, 2 – polished, 3 – electropolished
Rys. 8. Wyniki adhezji bakterii w stosunku do wartości chropowatości powierzchni: 1 – piaskowane, 2 – polerowane, 3 – elektropolerowane

CONCLUSIONS

The properties of the coating strictly depend on the method of surface preparation. For the selected process parameters, the best adhesion of the coating to the substrate was measured for sandblasted samples, as they had the highest surface roughness and the lowest wetting angle values of the tested variants. The observed change in coating continuity in coating scratch tests is highly dependent on a number of factors, such as the type of coating, its hardness, thickness and roughness, as well as the mismatch between coating hardness and substrate **[L. 25]**. The coefficient of friction of the coating, measured under a 5 N load, increased only for sandblasted samples, which may have been influenced by the strong adhesion of the coating to the substrate. For all variants, the coating slightly increased the wetting angle. Electropolishing is one of the methods used to increase the resistance of 316LVM steel to pitting corrosion **[L. 26]**, while the obtained polarization resistance values after coating indicate that the corrosion resistance is comparable to the mechanically polished variant. Titanium nitride affected the corrosion resistance of the all variants, R_p increased, while i_{corr} and E_{corr} decreased. The coating reproduced the surface well for sandblasted and mechanically polished samples, and reduced its roughness in the case of electropolished ones. Reducing surface roughness by applying a TiN coating has the effect of reducing the adhesion of bacteria E. Coli, but in the case of the mechanically polished variant, the coating does not affect either Sa or bacterial adhesion. In conclusion, the applied TiN coating for the electropolished and sandblasted samples reduced the surface roughness and bacterial adhesion and increased the value of the wetting angle. In all variants, the coating improved corrosion resistance but did not improve tribological properties.

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