

## Research Article

# Adhesion of *E. coli* Bacteria Cells to Prosthodontic Alloys Surfaces Modified by TiO<sub>2</sub> Sol-Gel Coatings

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The evaluation of the degree of bacteria *E. coli* adhesion to modified surfaces of the chosen prosthodontic alloys was presented. The study was carried out on Co-Cr (Wironit), Ni-Cr (Fantocer), and Fe-Cr-Ni (Magnum AN) alloys. Bare substrate as a control and titanium dioxide coated samples were used. The samples were placed for 24 hours in bacterial culture medium. After incubation period, a number of bacterial cells were evaluated by scanning electron microscope. The study revealed that modification of the alloy surfaces by titanium dioxide coating significantly decreases the amount of bacteria adhering to the surfaces and that additionally bare metal alloy substrates have a different degree of susceptibility to bacterial adhesion.

## 1. Introduction

In native physiological conditions of the healthy organism, the oral cavity contains opportunistic microorganisms, among which the most numerous are bacteria; however, fungi and protozoa can be also found. Moreover, the presence of viruses has been frequently confirmed in this environment. Bacteria colonize all natural structures (soft and hard tissue) of the oral cavity, that is, mucosa, gingivae, and teeth enamel. In the oral cavity, bacteria adhere easily to the hard structures, especially teeth, as well as prosthodontic appliances. Microorganisms which adhere to the natural and artificial surfaces and form colonies surrounded by polymeric extracellular matrix are called the biofilm or plaque. Biofilm can be formed on the teeth (as dental plaque), gingiva (as gingival plaque), and dentures (as denture plaque). The plaque composition and degree of colonization depend on its location in the patient's oral cavity, saliva composition, diet, natural cleansing mechanisms, and hygienic procedures.

Presence of the microorganisms in the oral cavity may lead to corrosive destruction (MIC—microbiologically influenced corrosion) [1] of metal prosthodontic restorative materials [2, 3]. Even titanium and its alloys, considered to be

corrosion resistant, can be colonised by bacteria, which may initiate corrosion [2, 4]. The adhesion of microorganisms to natural and artificial surfaces and formation of the biofilm could be also responsible for the serious diseases [5].

Metabolic activity of microorganisms is associated with an initiation and/or an increase in electrochemical and chemical processes that lead to corrosive destruction of materials and their alloys. Metabolic activity may also cause modification of the surrounding environment resulting in physicochemical changes [6–8]. Due to this, when choosing alloys used in dentistry, it is necessary to evaluate their interactions with biological objects (bacteria, proteins) in the oral cavity environment.

Therefore, taking into consideration pathogenic features of the bacterial plaque and its influence on the homeostasis of the oral cavity, the degree of bacterial accumulation on metal alloys used in prosthodontics should be examined. This is of high significance for persons having tooth losses, particularly patients additionally suffering from periodontal and oral mucosa diseases.

In case of the lowered organism immunity, when the host does not have enough antibodies, bacterial flora finds ideal

TABLE 1: Chemical composition of alloys used for examinations.

Alloy	Number of elements [%] wt.						
	Cr	Mo	Fe	Mn	Ni	Si	Co
Fantocer	24.79	8.89	1.33	0.12	63.13	1.57	0.17
Magnum AN	23.38	2.94	43.42	1.24	25.70	2.99	0.12
Wironit	30.52	4.73	0.11	1.56	0.06	0.82	62.21

conditions for excessive growth. This is of great importance for patients using dentures. For patients with partial tooth loss, some of the remaining parts of teeth are used as abutments for prosthodontic reconstruction and have to withstand a higher load induced by forces associated with chewing. The teeth are subjected to stresses and lateral movements, sometimes leading to overloading. The attachment apparatus, that is, the alveolar bone, the tooth, and periodontal ligaments supporting the tooth in the alveolus, has to withstand all these forces. This may be manifested by lengthening of the periodontal ligament, which results in the periodontal space widening. Bacteria accumulating around the abutment can contribute to the development of inflammation and additionally to the periodontal space widening, bone structure rarefaction, and finally to weakening of the attachment apparatus maintaining the tooth in the alveolus.

Metal alloys, with cobalt, nickel, chromium, and iron as basic components, are frequently used in prosthodontics. Although they possess a relatively low corrosive resistance as compared to noble metal alloys [9], they are widely applied due to economic reasons. To improve their corrosion resistance, different types of coatings (nitrides, carbides, oxides, carbonitrides, and others) are deposited on their surfaces [10–14]. Many scientific papers indicate that these coatings can decrease the bacterial adhesion to the alloy surface [15, 16]. The thickness and conditions of  $\text{TiO}_2$  coating annealing can cause diffusion of alloy elements from the substrate to coatings, which can influence coating properties, that is, chemical and phase composition [17]. Thus, it seems reasonable to investigate how  $\text{TiO}_2$  coatings affect the bacterial adhesion to the surfaces of biomaterials (e.g., different chemical compositions) commonly used in prosthodontic appliances.

## 2. Aim of the Study

The aim of this study was to determine the degree of bacterial adhesion to the modified surfaces of the chosen prosthodontic alloys coated with titanium dioxide by the sol-gel method.

## 3. Materials and Methods

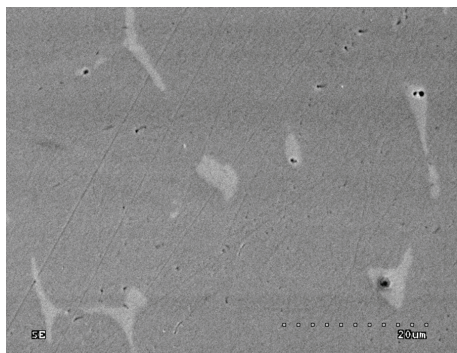
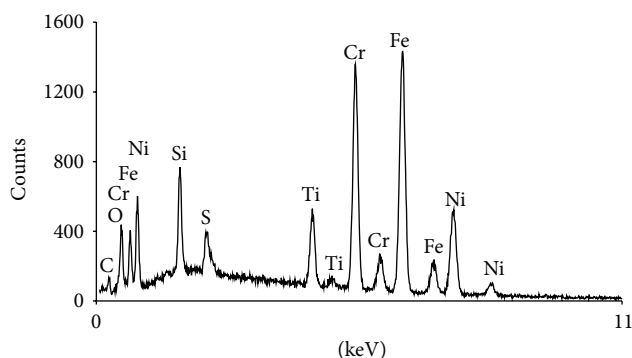
Cylindrical samples of selected alloys (Table 1) with a diameter of 8 mm and 5 mm height were used as a substrate in this study.

Ten samples were made from each alloy. They were divided into two groups. The samples of the first group were left uncoated (control samples), whereas those in the second group were coated with titanium dioxide.

$\text{TiO}_2$  coatings were elaborated with the sol-gel method. Titania sol was prepared from titanium (IV) butoxide by mixing it with absolute ethanol, acetic acid, and distilled water in a proper ratio. The samples were coated with the sol using the dip-coating method. The substrate was dipped and emerged from the sol with the speed of 30 mm/min. The dip-coated film was dried at room temperature for at least 15 min. Next, the sample was annealed at  $500^\circ\text{C}$  for 15 min. The process of the film deposition was repeated three times. The thickness of obtained coatings was about 300 nm.

The surface morphology and chemical composition of the prepared samples were examined by a scanning electron microscopy (SEM) using Hitachi S3000N microscope equipped with a Noran X-ray energy dispersive spectroscopy detector (EDS). The crystalline phase composition of  $\text{TiO}_2$  coatings was analysed with the scan step SIEMENS D-500 X-ray diffractometer (XRD) using  $\text{Co K}\alpha$  characteristic radiation and a graphite monochromator. An identification of phase composition was carried out with the use of the X-RAYAN computer software, supported with the ICDD database. For better clarity, XRD pattern of metallic substrate was subtracted from XRD pattern of  $\text{TiO}_2$  coating.

*E. coli* bacterial cells (DH5 $\alpha$  strain) were used as the biological material because *E. coli* is the most common microorganism used as a model organism. The investigation of bacterial surface colonization was carried out under flow condition of culture medium. The samples were ultrasonically cleaned. After that process, the samples were placed in a biological flow reactor chamber. An annular holder with holes was used to fix the samples. The rotational flow was forced by electromagnetic stirrer. The whole system with samples was steam autoclaved (Prestige Medical 2100 autoclave). Investigations were carried out at one level of rotational frequency of 150 rpm (150 rpm warranted laminar flow condition). At the next stage, the reactor was refilled with 200 mL of sterile culture medium. The Luria-Bertani (LB) medium was composed of Pepton G 0.5%, yeast extract 0.5%, and NaCl 1.0%. The samples were incubated for 24 h in a medium containing a standard number ( $340 \times 10^6$  per 1 mL) of *E. coli* (DH5 $\alpha$  strain) at  $37^\circ\text{C}$  under flow condition. After incubation, they were extensively washed with deionised water. This procedure allowed removing bacteria, which were not strongly adhering to the surface [18]. The samples, after the incubation period, were fixed for 1 h at  $4^\circ\text{C}$  with 2.5% glutaraldehyde in water. The fixed samples were then dehydrated with gradient series of ethanol, air-dried, and finally coated with a 20–30 nm-thick gold film in a sputtering apparatus (JEE-4X Jeol). Observations were made to count bacteria adhering to the sample surfaces. Scanning electron microscope was used to examine the specimens at magnification of 1000x and voltage of 5 kV. Thirty observation places were selected from each group of the studied samples, and the number of bacteria was evaluated for them. All procedures were repeated five times. The average cell number and standard deviation observed on the surfaces were used for the evaluation of colonization degree. The statistical parametric analysis based on small samples was applied to assess statistical significance of the results.

FIGURE 1: SEM image of TiO<sub>2</sub> coating on Magnum AN alloy.FIGURE 2: EDS analysis of TiO<sub>2</sub> coating on Magnum AN alloy.

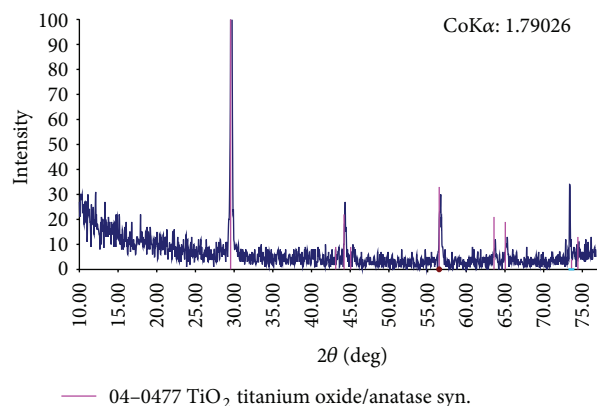
#### 4. Results and Discussion

The morphology of sol-gel TiO<sub>2</sub> coating on the dental alloy (Magnum AN) is shown in Figure 1. It can be observed that the coating well reproduced the microstructure of the metallic substrate surface. All investigated coatings were homogeneous with no visible signs of delamination, cracks, or flaking.

The spectrum of EDS elemental analysis of the previously-mentioned coating is shown in Figure 2. It contains elements originated from the substrate as well as titanium and oxygen originated from the coating, with the atomic ratio O/Ti = 2.

TiO<sub>2</sub> can exist as amorphous form or in several crystalline structures such as rutile, anatase, and brookite. The X-ray diffraction patterns of the TiO<sub>2</sub> sol-gel coating is shown in Figure 3. It was found that according to ICDD card 04-0477, TiO<sub>2</sub> revealed crystalline structure of anatase.

The example images obtained in the scanning electron microscope are shown in Figures 4(a) and 4(f). Figure 5 shows the chart presenting calculations of the number of bacteria adhered to the investigated surfaces. The data presented in Figure 5 demonstrate that the greatest amount of bacteria adhered to the unmodified alloy surfaces, amongst which Wironit alloy showed the higher amount of bacteria, while the lowest one was estimated in case of Fantocer. The additional relationship between nickel concentration in the alloy and the number of bacterial cells adhered to the surfaces

FIGURE 3: XRD pattern of TiO<sub>2</sub> coating.

was observed. Studied alloys evidently differed in the nickel content (Table 1). The negative correlation was found between the number of bacteria and nickel concentration (Figure 6). However, the similar amount of bacteria was detected on the surfaces of alloys coated with TiO<sub>2</sub>. Analogous results were found by Ciston et al. for zirconia ultrafiltration membranes coated with TiO<sub>2</sub> where the reduction in bacterial adhesion to the surface varied between 12 and 47% as compared to control samples [19].

Based on the ANOVA test and calculations of the number of bacteria adhering to particular samples, statistically significant differences ( $P < 0.0001$ ) between the groups were observed. The Bonferroni statistical test was used for evaluation of the statistical significance between all pairs of groups ( $P < 0.05$ ). However, there were no significant differences found in the number of bacteria that adhered to the surfaces of different alloys with deposited TiO<sub>2</sub> coatings (Table 2). Moreover, the number of adhered bacteria to the alloy surfaces with different Ni content was also statistically significant.

Titanium dioxide coatings have been found to reduce the number of bacteria adhered to their surfaces of about 37%–70% (compared to the bare substrates). Comparing the obtained results for TiO<sub>2</sub> coatings with those of alloy surfaces coated with titanium nitrides and carbonitrides [16], it can be stated that titanium dioxide coatings are not as efficient as TiN<sub>x</sub> and TiC<sub>x</sub>N<sub>y</sub>, regarding their resistance to bacterial surface accumulation. The amount of bacteria adhering to the surface was considerably lower for these coatings and equalled 0.7 per 1000 μm<sup>2</sup> for TiN, 1.9 per 1000 μm<sup>2</sup> for TiCN (containing 4% of carbon wt), and 2.4 per 1000 μm<sup>2</sup> for TiCN (containing 15% of carbon wt). However, it is difficult to univocally state that TiO<sub>2</sub> coatings are worse than titanium nitrides and carbonitrides regarding the resistance to the bacterial adhesion. Different conditions of performing experiments (TiO<sub>2</sub>—the flow culture, TiN, and TiCN—the stationary culture) might be the reason for such great differences. The culture in hydrodynamic conditions can better reflect the environment of the oral cavity. Moreover, the flow conditions allow better culture oxygenation and more even distribution of nutrients in the surrounding medium.



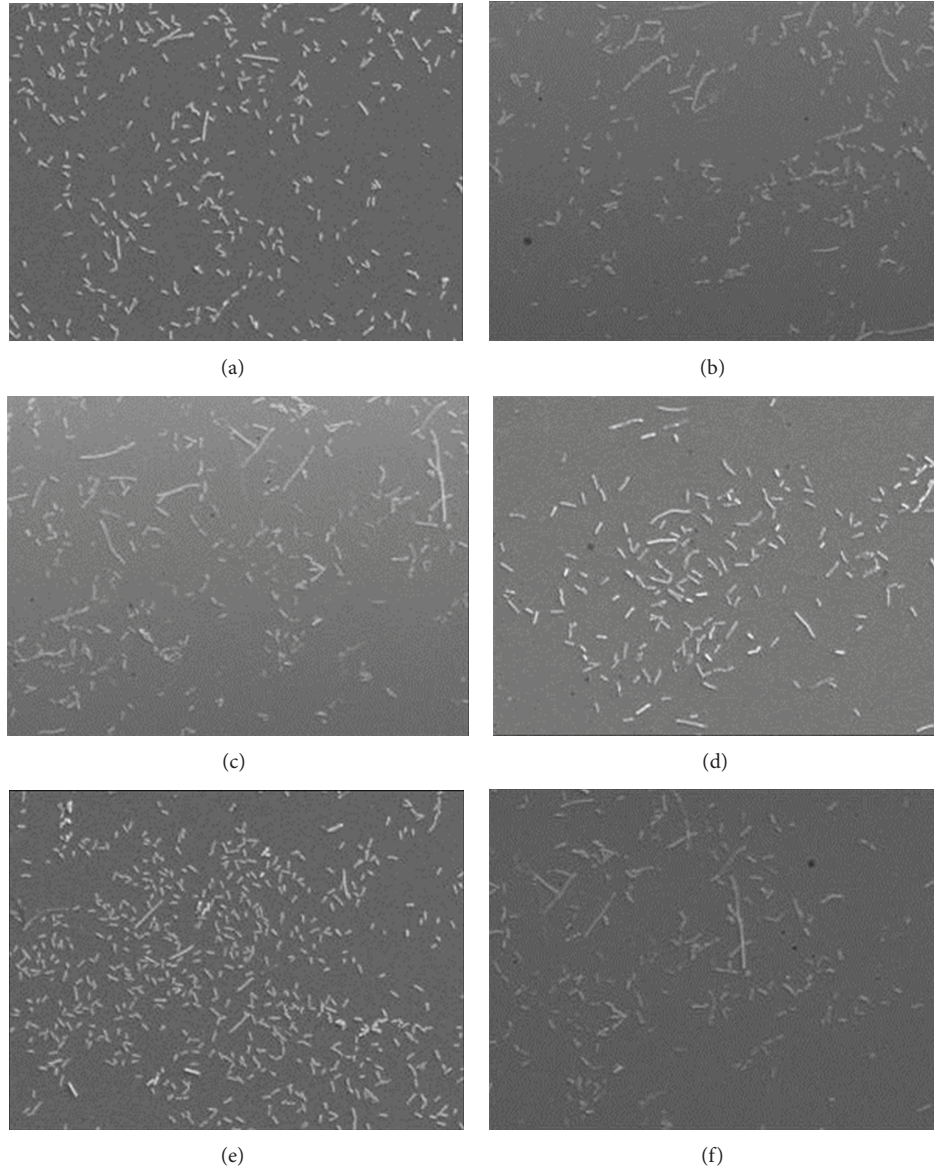


FIGURE 4: SEM pictures of the examined sample surface; (a) Fantocer; (b) Fantocer with  $\text{TiO}_2$  coating; (c) Magnum AN; (d) Magnum AN with  $\text{TiO}_2$  coating; (e) Wironit; (f) Wironit with  $\text{TiO}_2$  coating.

TABLE 2: The Bonferroni test between all pairs of tested groups ( $P < 0.05$ ).

Samples	Magnum AN	Fantocer	Wironit	Magnum AN with $\text{TiO}_2$ coating	Fantocer with $\text{TiO}_2$ coating	Wironit with $\text{TiO}_2$ coating
Magnum AN	—	+	+	+	+	+
Fantocer		—	+	+	+	+
Wironit			—	+	+	+
Magnum AN with $\text{TiO}_2$ coating				—	—	—
Fantocer with $\text{TiO}_2$ coating					—	—
Wironit with $\text{TiO}_2$ coating						—

Symbol “+” denotes results with statistical significance between couples of samples.

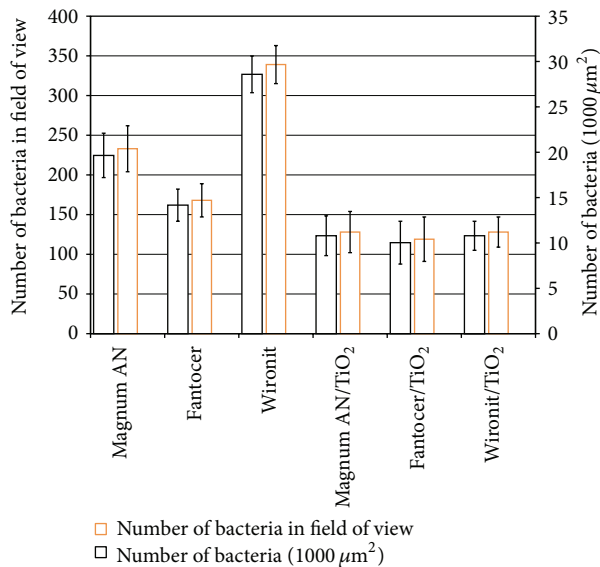


FIGURE 5: Number of bacteria adhered to the examined sample surfaces.

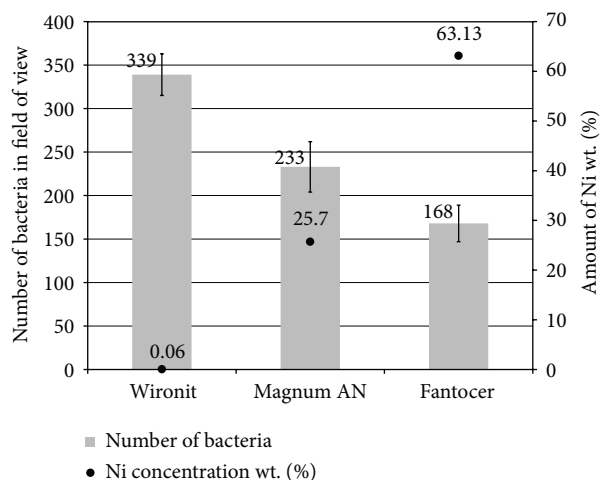


FIGURE 6: Relationship between the number of bacteria and nickel content in the alloy.

Better environmental conditions can induce intensification of bacterial adhesion.

TiO<sub>2</sub> coatings applied in different conditions may more effectively reduce microorganism adhesion due to their photocatalytic properties which cause the coatings to become bactericidal after ultraviolet radiation [19, 20]. The photocatalysis phenomenon might be applied in sterilization of medical instruments as well as during water and air purification [12–23]. However, this phenomenon is not significant in case of prosthodontic materials because of the way of their usage. These materials are kept in the oral cavity for a relatively long time, and free radicals formed due to the layer irradiation may lead to the impairment of cellular membranes, RNA and DNA [24].

Despite the fact that nickel reduces the number of bacteria accumulating on the alloy surfaces, it may induce allergic reactions of patients sensitive to nickel. Therefore, the deposition of protective coatings on alloy surfaces has a beneficial effect on the reduction of nickel ion release to the oral cavity tissues and the number of adhering bacteria.

## 5. Conclusions

- (1) Modification of prosthodontic alloy surfaces by TiO<sub>2</sub> sol-gel coating limits the biofilm formation compared to bare substrate.
- (2) Negative correlation between Ni content and bacterial colonization has been confirmed for selected alloys.

## Authors' Contribution

All authors cooperated in categories: (A) the preparation of the research program, (B) the execution of research, (D) the interpretation of data, and (E) preparation of the paper. Additionally, K. Banaszek and W. Szymański contributed in category (C) the statistical analysis.

## Acknowledgment

The term X-RAYAN is the name of the computer software made by SIEMENS intended to control and to process data obtained from diffractometer. There is no conflict of interests in the conducted studies and no financial gain.

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