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Antiadherent and antibacterial properties of TiO_2-coated and TiO_2:Ag-coated stainless steel orthodontic wires against *S. mutans* bacteria

JUSTYNA JOANNA BĄCELA[1]*, ZOFIA KIELAN-GRABOWSKA[2], BEATA BORAK[1], BEATA SOBIESZCZAŃSKA[2, 3], URSZULA WALCZUK[3], BEATA KAWALA[2], ANNA ZIĘTY[1], JERZY DETYNA[1], MICHAŁ SARUL[4, 5]

[1] Department of Mechanics, Materials and Biomedical Engineering, Faculty of Mechanical Engineering, Wrocław University of Science and Technology, Wrocław, Poland.
[2] Department of Dentofacial Orthopedics and Orthodontics, Wroclaw Medical University, Wrocław, Poland.
[3] Department of Microbiology, Wroclaw Medical University, Wrocław, Poland.
[4] Department of Integrated Dentistry, Wroclaw Medical University, Wrocław, Poland.
[5] Academic Dental Clinic, Wroclaw Medical University, Wroclaw, Poland.

Purpose: Conventional orthodontic treatment with stainless steel orthodontic wires may be detrimental to oral health, as it contributes to demineralized lesions and increases adhesion and bacterial biofilm formation, which contributes to cavity development. An alternative that has been investigated to reduce the side effects of orthodontic treatment is the use of coating materials with antimicrobial nanoparticles. This study aims to evaluate the antiadherent and antibacterial properties of TiO_2-coated and TiO_2:Ag-coated stainless steel orthodontic wires against *S. mutans* bacteria. *Methods*: In the sol–gel method, TiO_2:Ag thin films were deposited on stainless steel orthodontic wires. Coated archwires were analyzed for their antibacterial and antiadherent properties. The evaluation of *Streptococcus mutans* adhesion to the orthodontic wires' surface was conducted according to the type of coating used, biofilm formation assay, and measurement of the pH of the bacterial community. *Results*: In the microbiological test, the TiO_2:Ag coatings revealed a statistically significant difference in terms of microbial adhesion and biofilm formation by *Streptococcus mutans*. The TiO_2:Ag coating on stainless steel wire increased pH levels in the saliva environment. *Conclusions*: It can be concluded that antimicrobial orthodontic wires coated with silver-TiO_2 nanoparticles using the sol–gel thin film are a promising choice for improving orthodontic treatment.

Key words: *silver nanoparticles, titanium oxide, antibacterial, sol–gel dip-coating method, stainless steel orthodontic wires, antiadherent*

1. Introduction

It is well documented that untreated malocclusion may have a negative impact on quality of life and self-esteem, especially in a society where straight teeth and a beautiful snow-white smile are treated as a sign of success and happiness [17], [22]. The subjective sense of aesthetics is thus an important motivator to undertake orthodontic treatment and to maintain co-operation during treatment [34].

Currently, most malocclusions are treated with a fixed orthodontic appliance, the presence of which in the oral cavity creates an additional plaque retention area [28]. In modern orthodontic practice, a variety of metallic alloys are used: stainless steel, nickel-titanium, titanium-molybdenum and others. The choice of a suitable orthodontic wire is based primarily on its mechanical properties, as it largely determines its effectiveness [38].

Poor oral hygiene contributes to the formation of a metabolically active biofilm that affects the balance

* Corresponding author: Justyna Bącela, Department of Mechanics, Materials and Biomedical Engineering, Faculty of Mechanical Engineering, Wrocław University of Science and Technology, ul. Smoluchowskiego 25, 50-372 Wrocław, Poland. E-mail: justyna.bacela@pwr.edu.pl
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of demineralization and remineralization, which can lead to the formation of white spots on the enamel (enamel hypoplasia), caries, gingivitis and periodontosis as well as may change the mechanical properties of the appliance's elements [17], [35], [36].

The duration of treatment is also important – several years of orthodontic treatment influence the local harmful effect of bacterial metabolites on tooth enamel [32].

The growth of humus-forming and acid-forming bacteria, especially *Streptococcus mutans* and *Lactobacillus*, results in a decrease in the pH value of plaque to a level where remineralization is no longer possible. This causes enamel demineralization, formation of carious white spots followed by deep carious lesions and gingivitis. It is alarming that white spots and lesions around the orthodontic brackets were observed within 1 month from the start of treatment [15]–[18].

A promising strategy to reduce the degree of adhesion of bacteria to biomaterials is the use of coatings with bacteriostatic and bactericidal properties. Different coating material may be combined with dental materials, which is meant to reduce microbial adhesion and prevent caries [7].

In terms of various precious metals, silver has long been known for its antibacterial activity against Gram-positive (including cariogenic *S. mutans*), Gram-negative bacteria, fungi, protozoa and certain viruses, including antibiotic-resistant strains [16].

In the past decade, the antibacterial activity of some metal and metal-oxide films such as Ag, TiO_2, and ZnO has been investigated [6]. In terms of these materials, TiO_2 gained the greatest attention due to its specific properties. The biocompatibility of TiO_2 has been proven, and a large number of medical devices are made of titanium alloys. It is well documented that TiO_2 in the form of anatase is capable of oxidation and degradation of various organisms, including viruses, bacteria, fungi, algae and cancer cells. *In vitro* investigations of the antibacterial and antiadherent properties of TiO_2 have revealed less bacterial adhesion to TiO_2-coated surfaces [12], [29], [39]. Therefore, the use of photocatalytic and antiadhesive properties of TiO_2 in a clinical setting may be effective in terms of preventing bacterial adhesion and bacterial colony growth around orthodontic instruments. The TiO_2:Ag coatings were developed to achieve bactericidal properties more effectively. It has been shown that these coatings contribute to the disinfecting properties of TiO_2 anatase through the addition of silver [9], [39].

The physicochemical and mechanical properties of the TiO_2:Ag and TiO_2 coatings were previously investigated by Z. Kielan-Grabowska et al. [19]. The properties of obtained coatings were investigated using scanning electron microscopy, X-ray diffraction and electrochemical tests. Corrosion studies were performed in a Ringer's solution, which simulated physiological solution. Morphologies of the samples were observed using SEM and it showed TiO_2 and TiO_2:Ag coated wires were smooth and cracking as well as delamination of the coatings were not observed. Based on the results of the energy-dispersive X-ray spectroscopy (EDS), it was found that the grains observed in the SEM images are mainly constituted by TiO_2 with doping levels of Ag. It was also found that Ag aggregated into particles, but the particles were larger and much more dispersed in the film than the TiO_2 particles. XRD analysis found the presence of Ag nanoparticles, which indicated that the layer applied to stainless steel orthodontic archwires had antibacterial potential. For the base TiO_2 powder, peaks referring to the anatase phase with the main diffraction peak at $2\Theta = 25.25$ were observed. The temperature of 500 °C, which was used for calcination, suffices to start the rutile phase crystallization, as evidenced by the diffraction peak at $2\Theta = 27.35$. The XRD pattern of the modified powder shows no signs of rutile crystallization. The difference between the TiO_2 powder and TiO_2:Ag powder was the intensity of the peak near $2\Theta = 38°$, which, apart from the anatase phase, could refer to the main peak of metallic silver. There were also new peaks at $2\Theta = 43.5$, 64.3, and 77.25, which directly confirmed the presence of metallic silver in the powder. The study authors investigated the electrochemical behavior of TiO_2 and TiO_2:Ag-coated stainless steel orthodontic arches in comparison to uncoated 316L orthodontic arches in simulated body fluids. Open-circuit potential measurements (OCP) performed after 1 h. The obtained results for uncoated SS wires showed that the potential EOCP increased suddenly. This indicated the passivation of this material, which could also be seen at the next stage of electrochemical tests. Furthermore, the continued shift of potential to positive values indicated that there were changes in the passive layer. The potential equaled about −300 mV vs. SCE (saturated calomel electrode) compared to specimens of archwires with coatings, which could be evaluated as the initiation of localized corrosion or tendency to corrode. However, subsequent investigations revealed that these potential decreases might be related to cathodic inhibition. Moreover, a shift of EOCP towards positive values also for a SS archwire sample with a TiO_2 layer was observed. It indicated that this specimen could also form a passive oxide layer. It was observed that TiO_2 and TiO_2:Ag coatings exhibited a remarkable decrease

in corrosion resistance. Values of corrosion current density of the bare material were by four orders of magnitude lower than the corrosion current density for the coated samples. Another important parameter for determining the corrosion behavior of the samples was polarization resistance. While the R_p value for untreated SS archwire was 3.31 MΩ·cm^2, polarization resistance of TiO_2 and TiO_2:Ag coatings were measured as only 0.01 MΩ·cm^2 and 0.03 MΩ·cm^2. Corrosion potentials of the sample with TiO_2 and TiO_2:Ag coatings were almost similar and were equal to −270 mV and −332 mV, respectively. At the same time, E_{corr} for untreated archwires was 31 mV. It was observed that TiO_2:Ag coatings can change the microstructure and electrochemical behavior of stainless steel.

However, to date, the authors have not found a systematic review or meta-analysis that could prove the clinical effectiveness of such coatings on orthodontic wires. In order to undertake such research, it is necessary to gather an appropriate number of well-documented original research. This study aims to evaluate the antiadherent and antibacterial properties of TiO_2-coated and TiO_2:Ag-coated stainless steel orthodontic wires against *S. mutans* bacteria.

2. Materials and methods

2.1. Materials

2.1.1. Preparation of stainless steel orthodontic wires

The stainless steel orthodontic wires (Adenta GmbH) were the subject of this study. The cross-section of a sample in its initial state was rectangular and measured 0.016 × 0.022 inches. The orthodontic wires were cleaned in acetone and distilled water in an ultrasonic bath for 15 minutes [16]. The surface modification of stainless steel orthodontic wires coated with TiO_2:Ag was carried out using the sol–gel thin film dip-coating method. This can be explained in three parts.

2.1.2. Preparation of TiO_2 sol

The TiO_2 solution consisted of 6 ml of titanium(IV) isopropoxide (97%, Aldrich), 85 ml of isopropanol (Eurochem BDG, Poland) and 0.5 ml of acetic acid (99%, Aldrich). Polypropylene glycol (PPG, M_w = 1000, Alfa Aesar) at 1 wt. % was added to the TiO_2 solution in order to decrease the surface roughness. After adding PPG to the solution, it was stirred with a magnetic stirrer at room temperature for 3 hours. The stirred sol was aged for 24 h at 4 °C.

2.1.3. Preparation of nano-TiO_2:Ag sol

Silver nitrate ($AgNO_3$; Sigma-Aldrich) was used to prepare nano-TiO_2:Ag sol. One grams of $AgNO_3$ was dissolved in a mixture of 2.4 ml of water, 10 ml of acetic acid and 12 ml of isopropanol. Then the solution was mixed with the TiO_2 precursor sol at room temperature for 3 hours by means of a magnetic stirrer [19].

2.1.4. Preparation of thin films

Stainless steel wires were coated with TiO_2 and TiO_2:Ag films using the sol–gel dip-coating method. Each orthodontic wire was dipped in the sol for 1 minute and removed at a constant speed of 65.8 mm/min to obtain an even coating. The substrates were dried for 1 hour at 120 °C with a heating and cooling rate of 0.5 °C/min. This procedure was repeated two times to increase the thickness of the thin film. The number of the deposited coatings on the wire was 2 layers. The coated wires were then calcined for 2 hours at 500 °C with a heating and cooling rate of 1 °C/min in the laboratory atmosphere (ambient conditions). [19].

Straight parts of orthodontic archwires were cut into 1 cm long samples each after being annealed. The final subgrouping of the tested material is shown in Table 1.

Table 1. Groups of surface-modified orthodontic wires used for microbiological tests

Group 1	The control group consisted of uncoated stainless steel orthodontic wires
Group 2	Experimental group consisting of stainless steel orthodontic wires coated with TiO_2 thin film; base coating; "B"
Group 3	Experimental group consisting of stainless steel orthodontic wires coated with a thin TiO_2:Ag film; functional coating; "A"

2.2. Test method – microbiological part

2.2.1. Characteristics of tested bacterial strain and bacterial culture conditions

The coatings were tested for a reference strain of *S. mutans*, ATCC 25175. *S. mutans* was cultured for

12 hours in brain heart infusion (BHI) broth at 37 °C. One ml of an 18-hour streptococcal culture was centrifuged (16,000 rpm, 10 min., 4 °C), the bacterial pellet was suspended in phosphate buffer (PBS, pH 7.4), and the density of the bacterial suspension was determined spectrophotometrically to be 6×10^8 CFU (colony forming units)/ml. The *S. mutans* suspension prepared was diluted 1:100 in artificial saliva (Lab-lemco 0.05 g, yeast extract 0.1 g, protease peptone 0.25 g, mucin 0.125 g, NaCl 0.01 g, KCl 0.01 g, $CaCO_3$ 0.015 g/1 l) which was prepared according to Pratten et al. [33].

2.2.2. Evaluation of *S. mutans* adhesion to the orthodontic archwire coatings tested

The adherence and colonization of tested coatings by *S. mutans* were analyzed in artificial saliva. A sterile stainless steel uncoated and coated with the base coating (B) and functional coating (A) orthodontic archwires were pre-incubated in 1 mL of sterile artificial saliva at room temperature with gentle stirring on a bench rocker for 1 h to allow binding of mucins from artificial saliva on their surfaces. The saliva-dipped archwires were then infected with *S. mutans* suspension (6×10^8 CFU/mL) and incubated for 4 h at 37 °C under microaerophilic conditions, i.e., in an atmosphere with a 5–10% CO_2. After incubation, the orthodontic archwires were gently rinsed with 0.9% saline to remove loosely bound bacteria and placed into 1 mL of tryptose soy broth (TSB). Then, samples were sonicated on ice (3 × 5 sec. 20 kHz, Labo-Plus sonicator, Vibra Cell Sonics) to remove bacteria bound to the surface of the tested archwires. The resultant bacterial suspensions were diluted in TSB broth, and 100 µL of each dilution was plated onto tryptose soy agar (TSA) plates. The plates were incubated for 48 h at 37 °C under microaerophilic conditions. Then, *S. mutans* colonies were counted and the results were expressed as a number of bacterial colonies per 1 mL (colony forming units; CFU/mL). The number of bacterial colonies grown from the coated archwires was related to the number of colonies on the uncoated archwires, considered 100%. The test was repeated three times in triplicate, and the results are the average of the measurements.

2.2.3. Evaluation of biofilm formation by *S. mutans* using a quantitative culture method

S. mutans biofilm on the tested archwires was evaluated in artificial saliva after 24, 48, and 96 h of incubation at 37 °C, under microaerophilic conditions as in the adherence assay. After pre-incubation in artificial saliva, a sterile stainless steel uncoated and coated with the base coating (B) and functional coating (A) orthodontic archwires were infected with *S. mutans* and incubated at 37 °C under microaerophilic conditions. During incubation, the culture medium was replaced daily to neutralize the effect of its acidification during biofilm formation. In addition, the pH of the medium with samples was measured daily during the four-day incubation period with a pH meter (pH meter SeveenEasy, Mettler Toledo). After incubation, the infected archwires were washed and sonicated in 1 mL of TSB broth to remove bounded bacteria. The resultant bacterial suspensions were diluted in TSB broth and plated onto TSA agar plates to count bacterial colonies. The number of bacterial colonies grown from the coated archwires was compared to the number of colonies on the uncoated archwires, considered 100%. The test was repeated three times in triplicate, and the results are shown as the average of three experiments performed independently for three samples of each archwire.

2.2.4. Microscopic visualization of *S. mutans* biofilm on orthodontic wires

For biofilm visualization, orthodontic archwires incubated for 24, 48, and 96 h with *S. mutans* were fixed with 4% buffered formalin for 10 min, washed three times with phosphate buffer, and stained for 10 min with acridine orange (1 mg/mL) in phosphate buffer. After two washes, the archwires were inspected under a fluorescence microscope (Olympus BX51).

2.3. Statistical analysis

Statistical analyses were performed with the TIBCO Statistica package use in order to verify the hypotheses formulated in the study. This package was used for the analysis of basic descriptive statistics and the Shapiro–Wilk test, one-way analyses of variance (one-way ANOVAs), and Student's *t*-tests for independent specimens. The standard threshold of $\alpha = 0.05$ was considered the significance level. The Levene's test was performed to verify the homogeneity of the variance in the groups under study. Post hoc tests were performed using the Newman–Keuls test to accurately analyze and assess differences between the study groups. A series of one-way ANOVAs was performed in a between-group design. When the assumption of homogeneity of the variance between groups was not met, Welch's ANOVA was used.

3. Results

3.1. The basic descriptive statistics of the quantitative variables measured and the assessment of normality of distribution

First, basic descriptive statistics of the analyzed quantitative variables were counted. The normality of the distribution of the analyzed variables was checked using the Shapiro–Wilk test. In Table 2, the results of each test (pH level, adhesion test and formation of *S. mutans* biofilms) are presented. In this situation, one of the basic conditions for further parametric analyses (tests) has been met.

3.2. Analysis of the effect of coating on pH levels

In the next step of the analysis, it was checked whether the coating significantly affected pH levels. Post hoc tests were performed using the Newman–Keuls test to accurately analyze and assess differences between the study groups. The results of the post hoc test for each experimental group are shown in Table 3.

After 24 hours, there were significant differences between the modified archwires (with functional and

Table 2. Basic descriptive statistics for quantitative variables under study

	N	M	Me	SD	Sk.	Kurt.	Min.	Max.	W	*p*
pH										
t = 24 h										
A	8	6.07	6.07	0.20	1.36	2.46	5.86	6.49	0.855	0.099
B	10	5.93	5.95	0.13	0.01	–1.96	5.77	6.10	0.872	0.106
C	9	5.76	5.78	0.11	–0.33	–0.72	5.58	5.91	0.959	0.798
t = 48 h										
A	9	5.92	6.00	0.20	–0.89	–0.29	5.54	6.12	0.874	0.139
B	10	5.65	5.68	0.16	–0.37	–1.77	5.44	5.82	0.848	0.056
C	11	5.66	5.67	0.18	0.01	–1.70	5.44	5.90	0.883	0.115
t = 96 h										
A	6	6.24	6.26	0.31	–0.04	–3.22	5.91	6.53	0.753	0.021
B	8	5.94	5.93	0.05	–0.09	–1.37	5.86	6.00	0.947	0.681
C	8	5.99	6.00	0.04	0.15	–0.14	5.94	6.05	0.935	0.567
Cultures [CFU/ml]										
t = 24 h										
A	5	1846000	1300000	1398599.30	1.66	2.73	730000	4200000	0.829	0.138
B	5	1960000	2150000	926957.39	–0.16	–2.76	1000000	3000000	0.868	0.260
C	5	77280000	13400000	98548932.01	0.69	–2.78	1000000	204000000	0.775	0.050
t = 48 h										
A	5	62920000	53000000	39373303.14	0.27	–2.55	19500000	109600000	0.912	0.481
B	5	140500000	153000000	59524154.76	–1.00	1.93	46500000	208500000	0.933	0.622
C	5	230700000	268000000	90126716.35	–0.42	–2.91	126500000	323000000	0.858	0.224
t = 96 h										
A	5	299900000	260000000	149660616.06	–0.08	–1.84	107000000	458000000	0.906	0.450
B	5	143800000	130500000	37946673.11	0.60	–1.71	105000000	195500000	0.923	0.554
C	5	501900000	455000000	199377280.55	–0.34	–1.07	226000000	708500000	0.913	0.486
Adhesion [CFU/ml]										
t = 4 h										
A	7	188571.43	160000.00	102863.76	1.70	3.49	90000.00	400000.00	0.837	0.093
B	6	423333.33	410000.00	179071.68	0.27	–1.54	220000.00	670000.00	0.943	0.691
C	5	634000.00	610000.00	299299.18	1.31	1.90	360000.00	1120000.00	0.884	0.331

N – number of measurements; M – mean; Me – median; SD – standard deviation; Sk. – skewness, Kurt. – kurtosis; Min and Max – lowest and highest value of the distribution; W – Shapiro–Wilk test result; *p* – significance level; A – functional coating (archwires with $AgNO_3$); B – base coating (archwires with a base coating); C – original state (SS steel – uncoated archwires = C; $p < 0.05$ is marked in bold.

Table 3. Newman–Keuls post hoc test results for the dependent variable of pH levels

t = 24 h			
Group	{1}	{2}	{3}
A {1}		0.0678	**0.0007**
B {2}	0.0678		**0.0236**
C {3}	**0.0007**	**0.0236**	
t = 48 h			
Group	{1}	{2}	{3}
A {1}		0.0067	**0.0035**
B {2}	0.0067		0.8958
C {3}	**0.0035**	0.8958	
t = 96 h			
Group	{1}	{2}	{3}
A {1}		**0.0058**	**0.0084**
B {2}	**0.0058**		0.5474
C {3}	**0.0084**	0.5474	

Significant differences for $p < 0.05$ are marked in bold.

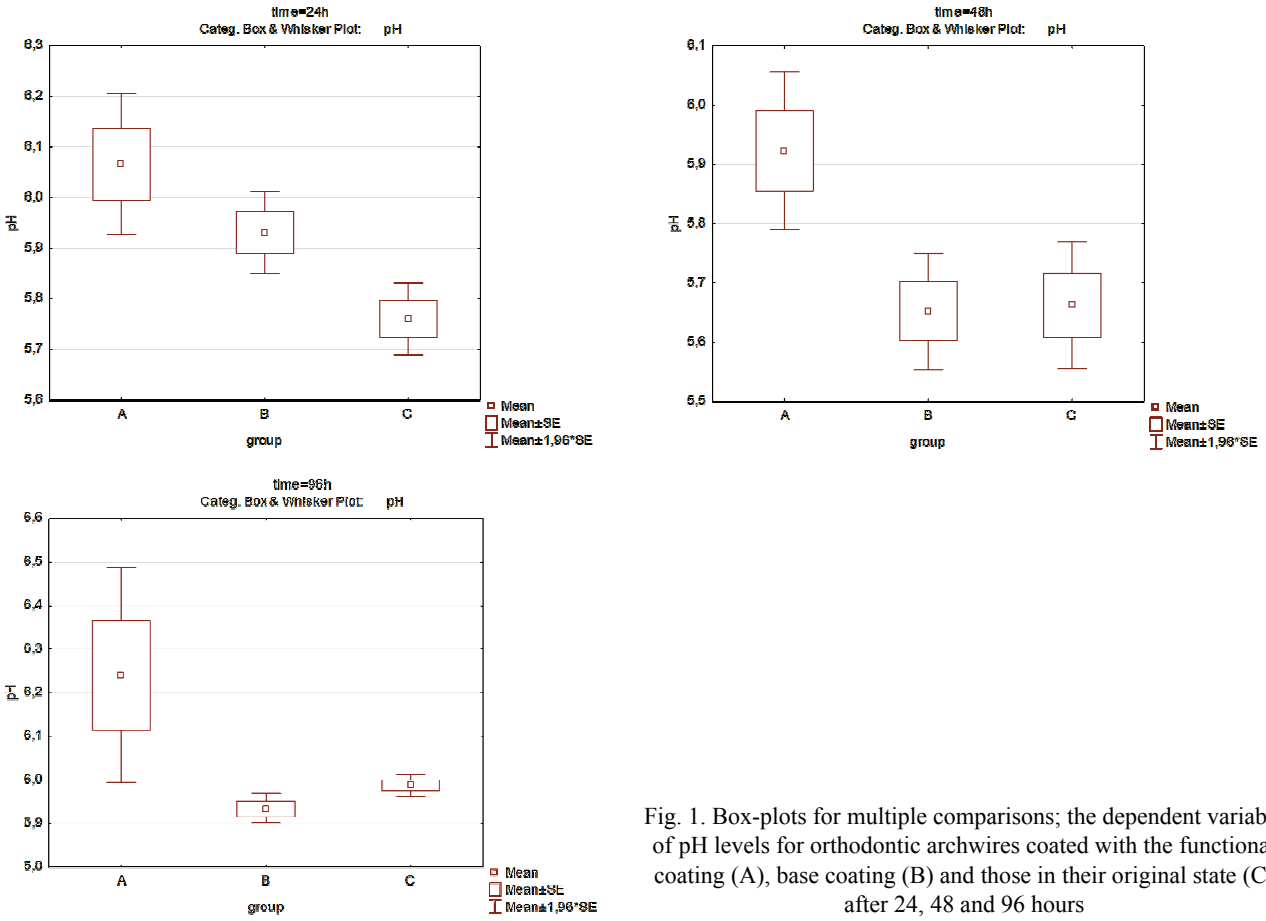

Fig. 1. Box-plots for multiple comparisons; the dependent variable of pH levels for orthodontic archwires coated with the functional coating (A), base coating (B) and those in their original state (C) after 24, 48 and 96 hours

base coatings) and the reference sample (without coating). After prolonged testing (48 h and 96 h), the sample coated with the A coating induced significantly higher pH levels in the saliva environment compared to the archwire coated with the B coating and that without the coating. The level of significance for rejecting the null hypothesis is very high in each of the comparisons indicated.

The differences between the study groups described above are well illustrated by box-plots in Fig. 1. After 24 h, the TiO_2:Ag coating increased the pH value of the saliva environment by 5%, compared to the reference sample, after 48 h and 96 h by 4%.

3.3. Analysis of *S. mutans* adhesion to the test surface

An important parameter of orthodontic archwires is their antiadhesive surface for microorganisms colonizing the oral cavity. This level was evaluated after 4h of experimentation. Newman–Keuls post hoc test results are shown in Table 4.

Table 4. Newman–Keuls post-hoc test results for bacterial cultures

$t = 4$ h			
Group	{1}	{2}	{3}
A {1}		0.0590	0.0041
B {2}	0.0590		0.0866
C {3}	0.0041	0.0866	

Significant differences for $p < 0.05$ are marked in bold.

Strong significance can be observed for the difference in terms of the means between groups A and C (significance level far from the threshold) in favour of the group with A coating. It should also be noted that the differences between the other groups in this experiment are significant but not substantial.

As mentioned above, the bacteria adhered much less well to the sample coated with the A coating compared to the reference sample (without coating – C). At the same time, in Fig. 2, it is shown that the biofilm on A coating is loose, fragmented and prone to detachment, whereas the biofilm on uncoated C archwire forms three-dimensional clusters between the layer of adherent bacteria that do not contain clustered (Figs. 2A, B, C). This suggests the ease of detachment of biofilms from the A-coated archwire compared to the compact biofilms found on the uncoated archwire.

This may also explain the higher bacterial counts on the A coating compared to the B coating, on which the biofilm was more compact, making it more difficult to disintegrate during sonication (Fig. 2). After 4 hours, the A coating reduced the adhesion of the *S. mutans* bacteria to the wire surface by 74% while the B coating by 33%.

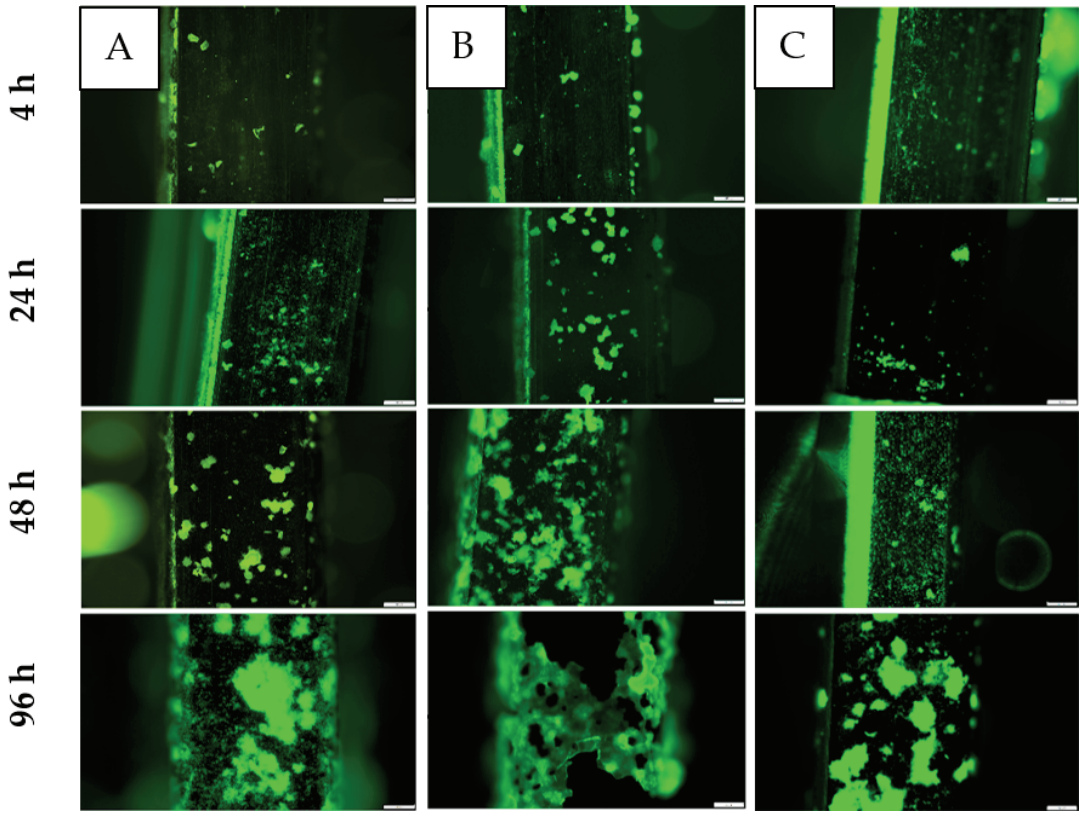

Fig. 2. Surface observation of specimens A, B, and C using an Olympus BX51 fluorescence microscope. Functional coating (A) , base coating (B), uncoated surface (C)

3.4. Analysis of the effect of orthodontic archwire coatings on the formation of *S. mutans* biofilms

In the next step of the analysis, it was verified whether the result of the cultures is dependent on the applied coating on the surface of the orthodontic archwires. Newman–Keuls post-hoc tests were conducted to assess significant pairwise differences. The results of the Newman–Keuls test are summarized in Table 5.

Table 5. Newman–Keuls post-hoc test results for bacterial cultures

$t = 48$ h			
Group	{1}	{2}	{3}
A {1}		0.0895	**0.0048**
B {2}	0.0895		0.0529
C {3}	**0.0048**	0.0529	
$t = 96$ h			
Group	{1}	{2}	{3}
A {1}		0.1159	**0.0488**
B {2}	0.1159		**0.0058**
C {3}	**0.0488**	**0.0058**	

Significant differences for $p < 0.05$ are marked in bold.

Almost all study groups are significantly different (high level of rejection of the null hypothesis between group A and C; between group B and C there is a significance level of 0.0529, i.e., on the borderline).

The A coating has significantly reduced bacterial growth. The described variations between the test specimens when divided into time groups can be seen in box-plots.

Biofilm analysis after 48 h clearly reveals that almost all study groups are significantly different (high level of rejection of the null hypothesis between group A and C; between group B and C there is a significance level of 0.0529, that is, on the borderline).

The findings indicate that the A coating significantly reduced the growth of bacterial biofilm. The described differences between the test specimens when divided into time groups can be seen in box-plot in Figs. 2 and 3.

The TiO_2:Ag coating reduced biofilm formation on the orthodontic archwire surface by 98% after 24 h, 73% after 48 h and 40% after 96 h, compared to the reference sample. On the contrary, the TiO_2 base coating reduced biofilm formation on the orthodontic archwire surface by 97% after 24 h, 39% after 48 h and 71% after 96 h compared to the reference sample, as shown in Fig. 4.

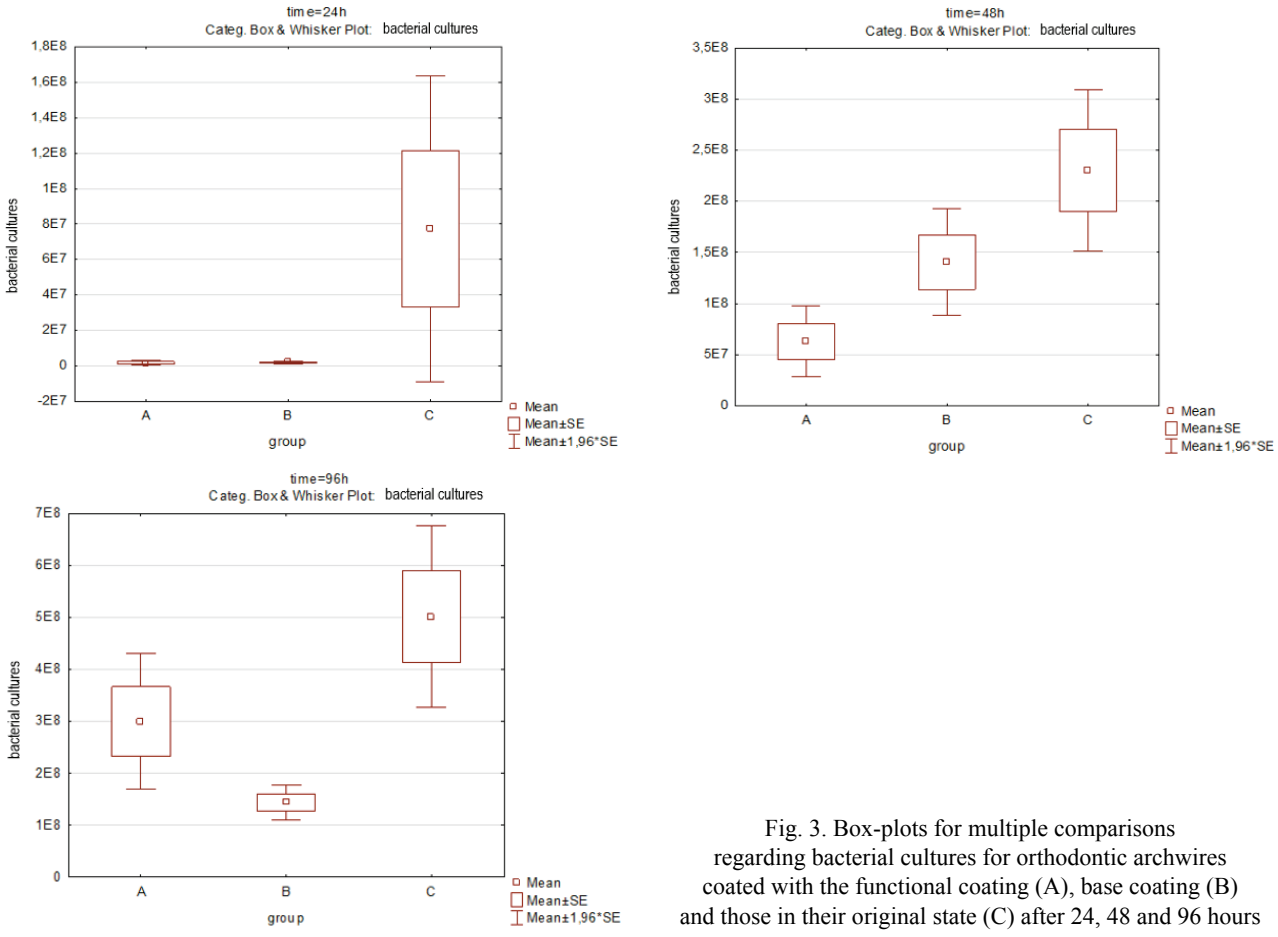

Fig. 3. Box-plots for multiple comparisons regarding bacterial cultures for orthodontic archwires coated with the functional coating (A), base coating (B) and those in their original state (C) after 24, 48 and 96 hours

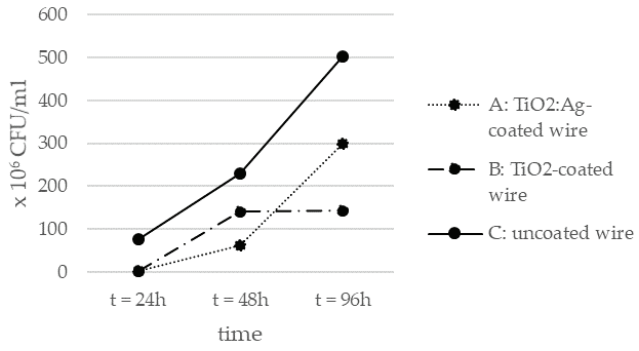

Fig. 4. Plot of the bacterial survival count on samples coated with functional coating (A), base coating (B) and on reference samples (an uncoated orthodontic wire – C) according to time

4. Discussion

Enamel demineralization and periodontal disease that develop during orthodontic treatment can affect even 50% of patients [5], [24], [28]. Marcusson et al. [27] found an increase in carious white lesions from 7.2% before treatment to 24–40.5% after treatment according to the type of dentine bonding agent used. It is alarming that white spots and lesions around orthodontic brackets were observed even within 1 month from the start of treatment [5], [14]. Furthermore, the authors proved in previous studies that the oral environment, including the impact of bacterial biofilm, can significantly alter the properties of orthodontic wires and thus affect the effect of therapy [19], [35], [36].

A promising strategy for reducing the degree of bacterial adhesion to biomaterials is the use of coatings that exhibit bacteriostatic and bactericidal properties. Coatings composed of nanoparticles are particularly noteworthy. Nanoparticles are insoluble particles that are less than 100 nm in size. Bacteria are less likely to develop resistance to metal nanoparticles compared to conventional antibiotics.

In recent years, there have been reports of studies related to the coating of parts of fixed appliances with bioactive coatings. The authors of these studies focused on coating various parts of fixed appliances [20], [26], [30]. It is also mentioned in their articles that various coating methods such as thermal vacuum evaporation [28], a thin film deposition method, physical vapor deposition (PVD) [2], [13], [29] hydrothermal synthesis or, as in the case of this paper, the sol–gel thin film dip-coating method [8], [10].

The authors of the present Paper initially attempted to use the methods described in various publications to cover orthodontic wires made of 18/8 chromium-nickel steel [8], [10], [23]. Unfortunately, none of the attempts to make such a coating, according to the descriptions presented in these publications was successful. The obtained coatings did not cover the wires tightly or did not adhere permanently to the wire surface. For this reason, the authors have tried to describe their own procedure for executing such shells, which was previously described [19]. After obtaining such a coating, however, it was necessary to confirm the biological effectiveness of such a layer.

In this study, as mentioned above, the surface modification of stainless steel orthodontic archwires was performed using the sol–gel thin film dip-coating. A series of detailed microbiological tests was conducted to confirm or exclude the antimicrobial activity of the A coating: the ability of *S. mutans* to adhere and colonize the tested coatings, the ability of *S. mutans* to form a biofilm on the tested coatings, and to observe the changes in pH levels of artificial saliva in the case of biofilm formation of *S. mutans* on individual coatings. The adherence and colonization of tested coatings by *S. mutans* were analyzed in artificial saliva. The saliva-dipped archwires were infected with *S. mutans* suspension (6×10^8 CFU/mL) and incubated for 4 h at 37 °C under microaerophilic conditions, i.e., in an atmosphere with a 5–10% CO_2. *S. mutans* biofilm on the tested archwires was evaluated in artificial saliva after 24, 48 and 96 h of incubation at 37 °C, under microaerophilic conditions, as in the adherence assay. Microbiological procedures were similar to those used by other researchers [3], [31]. Since publications differ in terms of incubation time, so in this study authors decided to examine the biofilm formation at three time intervals – after 24, 48 and 96 hours.

The obtained results confirmed antibacterial properties of the tested coating. The tested coating reduces *S. mutans* adhesion to the archwire, reduces biofilm formation on the surface of the archwires, increases pH levels of the saliva environment (compared to the reference archwires – without coating) and thus reduces bacterial colonization. Considering that, the tested coating may reduce the development of plaque and caries during orthodontic treatment. Lactic acid, the main virulence factor contributing to dental caries, is the major end-product of glycolysis by *S. mutans* at low environmental pH. The increased pH level of *S. mutans* cultures on TiO_2:Ag coating indicates that the coating lower environmental acidification by inhibiting lactic acid production.

It should be noted that the use of archwires with an antibacterial coating is not a substitute for mechanical removal of biofilm during proper tooth brushing, while the tested coating may much better eliminate the

local decaying effect of bacterial biofilm in people who take care of their hygiene compared to those who neglect this daily hygiene practice.

In a 2015 study, Mhaske et al. [28] evaluated the antiadherent and antibacterial properties of stainless steel orthodontic archwires and surface-modified with silver NiTi against *Lactobacillus acidophilus*. Although a different testing method and a different bacterial strain were used in that study, there was a reduction in the number of bacteria deposited on the archwires, as in this study. The antibacterial activity of applied coatings against *Lactobacillus acidophilus* was also studied by Shah et al. [37] and Cao et al. [8]. In both publications, the authors evaluated the antiadhesive and antibacterial properties of orthodontic brackets that were surface-modified with photocatalytic titanium oxide (TiO_2). In both studies, although different coating methods and different materials were used for orthodontic brackets, the TiO_2 coating was found to have antiadherent and antibacterial activity against *Lactobacillus acidophilus*.

Ghasemi et al. [13] tested the antibacterial activity of stainless steel orthodontic brackets coated with silver and titanium oxide nanoparticles. 55 stainless steel brackets were divided into 5 groups of 11 brackets each: uncoated brackets, brackets coated with a 60 mm silver layer, 100 mm silver layer, 60 mm titanium layer and 100 mm titanium layer. The coatings were made using the physical vapor deposition method. *S. mutans* colonies were counted 3, 6, 24 and 48 hours after contact with coatings. The authors' findings confirmed the results of their own study and revealed that all four coatings may reduce bacterial accumulation compared to the control group in which bacterial counts increased exponentially.

Antibacterial and antiadherent properties against *Streptococcus mutans* of TiO_2:Ag coating on orthodontic brackets was proven also by other researchers Fatani et al. [12], Liu et al. [21] and Arkusz et al. [4]. The results obtained in the present study allowed us to state that the antibacterial effectiveness of the chosen layer covering the orthodontic wires to be similar to that obtained by other authors for the layers covering the brackets. This is important because the geometric and mechanical properties of the wires are different from orthodontic brackets.

Furthermore, orthodontic wires provide a good environment for oral microorganisms, and, therefore, adherence of oral bacteria to fixed appliances can lead to wire damage and soft tissue inflammation [11]. Obtaining a tight, adherent layer on orthodontic wires requires a different technological approach and does not have to result in obtaining a layer of similar tightness and effectiveness. However, the authors' research proved that the presented wire coating technology allows for obtaining a layer of similar biological effectiveness *in vitro* as coating of orthodontic brackets. The effectiveness of the presented method of covering the wires was also proven by means of appropriate mechanical tests.

This study investigated the role of two different TiO_2 and TiO_2:Ag coated wires and the wire in its original state. The benefits of two orthodontic coated wires were compared in terms of adhesion, antimicrobial activity and pH. Modified surfaces of stainless steel supports coated with the TiO_2:Ag coating revealed a significantly reduced adhesion of major pathogens, *S. mutans*, that constitute biofilm in the oral cavity. The combination of TiO_2 with silver ions enhances the antibacterial activity of the coting. Aqueous suspensions of TiO_2 after exposure to visible light generate hydroxyl radicals (•OH) and superoxide anions ($\bullet O_2^-$), which significantly impact *S. mutans* biofilms, but are not toxic to host cells. According to Ahn et al. [1], *S. mutans* biofilm is dramatically reduced in the presence of oxygen which downregulates several genes involved in the biofilm formation. On the other hand, silver ions interact with thiol groups of enzymes in membranes and cytoplasmic proteins, and induce DNA strands breakage, thus inactivating bacteria. Similarly, the hydroxy radicals from TiO_2 oxidize thiol groups in enzymes [25].

However, the analysis of the presented results allows us to give a direct answer to the question of which layer has a stronger antibacterial effect. The results directly indicate that the antimicrobial activity of the resulting coating is a derivative of the presence of the TiO_2:Ag coating. The coating B (TiO_2) after 96 hours shows a lower number of bacteria counted after the sonication treatment. As it has been described, however, it may be a consequence of the much weaker adherence of the biofilm to the functional coating – A (TiO_2:Ag), and thus easier separation of colonies during the sonication treatment. This is confirmed in the descriptive analysis of the biofilm formed, based on microscopic photos. This thesis is also strongly supported by the analysis of environmental pH changes for wires covered with various coatings. The significantly higher pH of the environment in the group of wires covered with a layer of TiO_2:Ag (functional layer A), compared to both other groups, proves that bacterial metabolism is significantly preserved due to the presence of Ag ions in the active layer. This effect was noticeable after 24, 48 and 96 hours of incubation. Such an effect was not observed for the other layers.

5. Conclusions

The presented method turned out to be effective in the case of applying a layer on orthodontic wires. The obtained coating showed an effective *in vitro* antibacterial activity against *S. mutans*. The presented method proved to be similarly effective to coatings obtained by other researchers on orthodontic brackets.

1. After 4 hours, the TiO_2:Ag coating reduced the adhesion of *S. mutans bacteria* to the wire surface by 74% while the base TiO_2 coating – by 33%. The TiO_2:Ag coating on stainless steel wire showed antiadherence properties against the major oral pathogens, *S. mutans*.
2. The TiO_2:Ag-coated archwires have proven antibacterial properties, thus, indirectly, it may prevent caries and plaque accumulation.
3. Stainless steel wires coated with TiO_2:Ag can be used in orthodontics as they provide suitable antimicrobial activity and resistance to biofilm formation.
4. The TiO_2:Ag coating increases pH levels in the saliva environment, which can reduce the harmful effects of bacterial biofilm on parts of fixed appliances and patient tissues, preventing the development of local carious lesions.

The coating requires further testing before applying it to the oral environment. Longer times of adhesion and biofilm forming tests should be performed. The next step should be to investigate the potential cytotoxicity of the coated arches. If the absence of cytotoxicity is confirmed, biocompatibility tests and *in vivo* tests can be started.

Author contributions

Conceptualization, J.D, M.S.; method, J.B., Z.K.-G., A.Z.; validation, J.B., Z.K.-G., B.S., U.W.; formal analysis, J.B., Z.K.-G., B.B., J.D..; investigation, J.B., B.S., U.W.; resources, J.B., B.B., Z.K.-G., U.W., B.S.; writing – original draft preparation, J.B., Z.K.-G.; writing – review and editing, B.B., J.D., M.S.; visualization, J.B., Z.K.-G.; supervision, M.S., J.D., B.K., B.B., B.S., U.W.; project administration, J.D., M.S.; funding acquisition, J.D., M.S.

J.B. and Z.K.-G. contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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