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Influence of artificial saliva compositions on tribological characteristics of Ti-6Al-4V implant alloy

EDYTA ANDRYSEWICZ[1], JOANNA MYSTKOWSKA[1]*, JOANNA KOLMAS[2], MAREK JAŁBRZYKOWSKI[1], RAFAŁ OLCHOWIK[1], JAN R. DĄBROWSKI[1]

[1] Department of Materials and Biomedical Engineering, Białystok University of Technology, Białystok, Poland.
[2] Department of Inorganic and Analytical Chemistry, Medical University of Warsaw, Poland.

The present paper describes the results of tests on the influence of human saliva and its substitutes on tribological characteristics of implant materials on the example of the Ti-6Al-4V (a-Ti) titanium alloy. The saliva substitutes were prepared on the basis of pyrophosphates and mucins dissolved in saline buffer. The results of the presented tribological tests show that the values of the parameters under research varied from each other, while much similarity was observed between the evaluated level of wear characteristics after the friction process in the human saliva environment and that in the environment of one of the mucins tested. The microscopic observations of surfaces of the a-Ti samples after friction revealed varied forms of tribological wear. Infrared microspectroscopy studies of surfaces of the a-Ti samples after friction revealed the presence of secondary lubricating films based on mucin found in the artificial saliva solutions.

Key words: *friction coefficient, human saliva, linear wear, saliva substitute, Ti-6Al-4V alloy*

1. Introduction

In recent years, a dynamic cooperation of medical and technical circles, pertaining to developing new biomaterials for medical purposes, has been observed. This concerns, for example, dental surgery and prosthetic dentistry [1], [2]. Among others, metallic materials have been used there for a wide range of applications, including reconstructing or replacing missing teeth, or for correcting and sustaining the masticatory apparatus functions. Noble metals (gold, platinum) as well as palladium-, cobalt-, chromium-, and titanium-based alloys are the most commonly used materials [1], [3]–[5]. An example of overlay denture with such retentive elements is presented in figure 1.

Successful dental treatment depends significantly on the proper selection of metallic biomaterials with appropriate mechanical and physicochemical properties [3]. It is important due to the fact that these materials are required to be used and exposed to the influence of many factors, including e.g., human saliva, for many years.

Natural saliva which performs a number of significant functions in the human body, can also cause many health problems in patients (e.g., hyposalivation – insufficient production of saliva, or xerostomia – dryness of the mucous membrane of the mouth). Therefore, it seems that using commercial saliva substitutes might significantly influence the durability, reliability, as well as the functional quality of prostheses, thus influencing also the comfort of using prostheses by patients. Hence, commercial saliva substitutes should increase patients' life comfort and at the same time they should favourably influence the utilization properties of metallic biomaterials used in the oral cavity [6]–[10].

* Corresponding author: Joanna Mystkowska, Department of Materials and Biomedical Engineering, Białystok University of Technology, ul. Wiejska 45C, 15-351 Białystok, Poland. Tel.: 85 746-92-51, fax: 85 746-92-48, e-mail: j.mystkowska@pb.edu.pl
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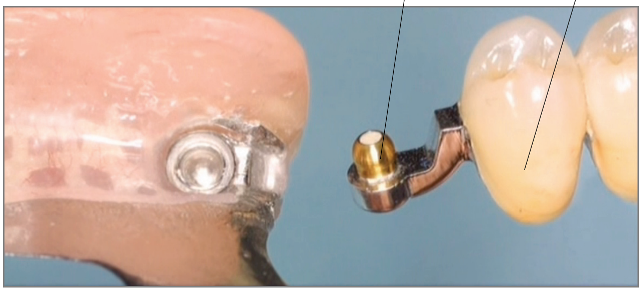

Fig. 1. Overlay denture retentive elements [5]:
1) based on Ti-alloy, 2) based on Ti-alloy + TiN coating

Numerous examples of research on different properties of saliva are the basis for developing saliva substitutes. Another important factor in this research is the influence of saliva and its substitutes on the durability of metallic materials which are to be applied in the oral cavity [4], [5], [11].

Research concerning development of new saliva substitutes has been continuously carried out for many years now [12], but the preparations obtained are, unfortunately, far from the original. Patients complain about short-lasting effects of these compositions, their inappropriate taste and consistency, and also about their low positive influence on facilitating the processes of speaking, swallowing, and chewing food.

The aim of the presented research was to evaluate in vitro the influence of natural human saliva and of the authors' own compositions of saliva substitutes on the tribological properties of implant materials on the example of the alloy which is most frequently used in implantology [4], [13], i.e., the two-phase a-Ti alloy. The second stage of the research involved investigating the a-Ti alloy surfaces after the tribological tests were carried out, in order to check whether lubricating film had been formed there. In literature there is little data concerning tribological tests of titanium alloys used as precise elements in dental prostheses [14]–[16]. In this work, the wear of attachment precise elements in dental prostheses, adapted in prostheses fixed on implants was evaluated. These elements are prepared of polymers or different metal alloys [11] and they operate in saliva environment under loading, which adds up to its wear.

In the study described, the FTIR microspectroscopy was used to analyze the chemical composition of the film. The Fourier transform infrared spectroscopy (FTIR) has been widely applied to detect the vibration characteristics of chemical functional groups and to study chemical structures of various materials, e.g., polymers, biomaterials, glasses, pharmaceuticals, and biological tissues [17]–[19]. Infrared microspectroscopy used in the transmission, reflection, or attenuated total reflection modes, is a powerful technique for obtaining chemical information from very small sections of samples. In the present paper, the FTIR microspectroscopy data obtained from the research on the mucin film formed on titanium surfaces is presented.

2. Materials and methods

In the present experimental research, human saliva was used besides three saliva substitutes whose compositions had been developed in the Department of Materials and Biomedical Engineering of the Białystok University of Technology. The saliva substitutes were selected on the basis of their wide use in the pharmaceutical industry (toothpastes, mouth rinses, etc.) [12]. The first composition contained three types of pyrophosphates as well as xanthan gum, while the second and the third compositions contained natural animal mucins of type II and type III, respectively. All the solutions were prepared on the basis of the PBS (phosphate buffered saline) solution.

In order to provide repeatable test conditions for natural saliva, the previously developed methods of collecting saliva samples were applied [20].

Tribological tests were conducted with the use of a T-11 type pin-on-disc tester. The tribological pair was composed of a-Ti pin whose tip was a truncated cone with the slant height angle of 20° and the initial truncated area of approximately 1 mm^2 (as the sample). Such sample geometry makes it possible to obtain great unit pressures at low normal load. The countersample was an a-Ti disc whose diameter was 25.4 mm. Figure 2 shows a laboratory stand and a diagram of the friction node.

Table 1 presents information on the materials and lubricants used in the tribological tests described.

On the basis of the initial research performed and literature review, the following parameters were adopted for the tribological tests: friction velocity, $v = 0.1$ m/s; friction radius $r = 8$ mm; countersample (disc) rotational velocity $n = 120$ rev/min; diameter of the truncated area of the sample $\phi = 1$ mm; loading force applied to the sample $F = 15.7$ N; time of friction in a single test $t = 3$ h (10800s); lubricant volume $V = 5$ cm^3.

The results of the tribological tests were statistically processed using the STATISTICA software package, StatSoft company. All the characteristics observed are the mean values obtained from three

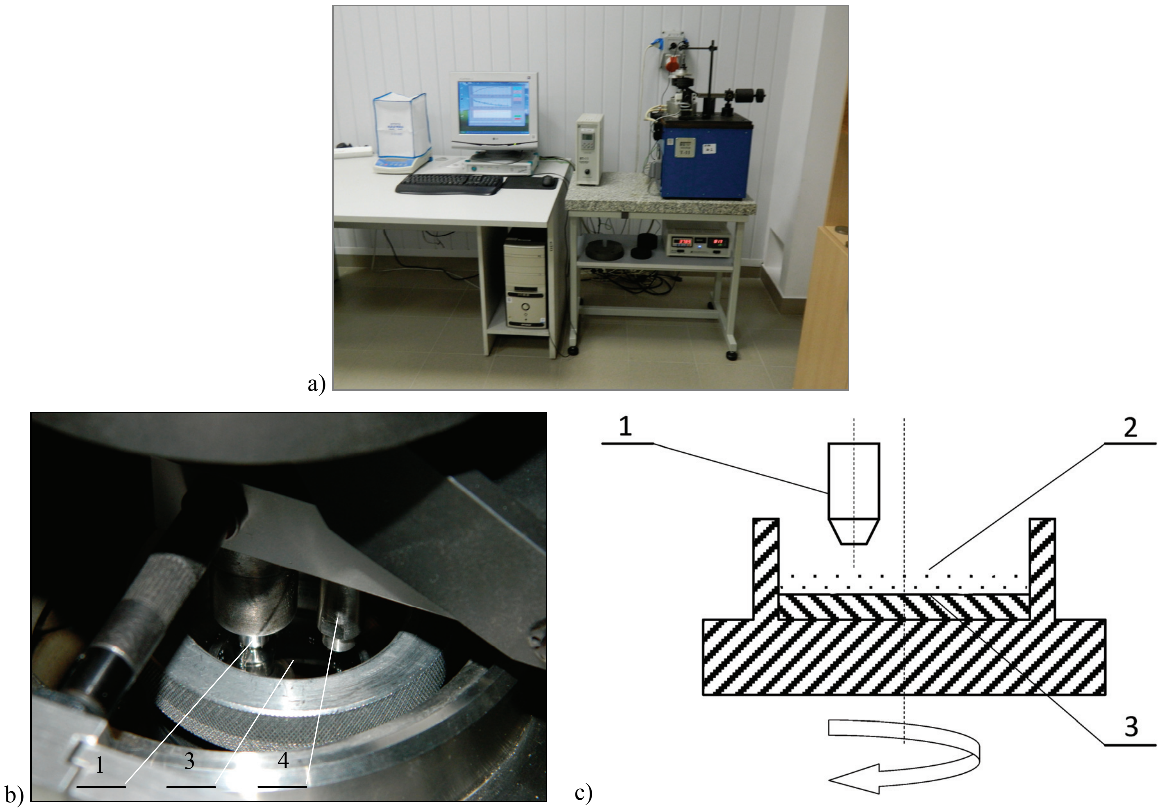

Fig. 2. A view of laboratory stand: (a) general, (b) friction node, (c) diagram of the friction node: 1,3 – a-Ti sample and countersample, 2 – lubricant, 4 – lubricant dropper

Table 1. Materials and lubricants used in the tribological tests

Friction nodes	Materials
pin (1)	• cylinder-shaped samples of Ti-6Al-4V alloy; *Ra* = 0.4 [µm] (produced by ChM Sp z o.o. Company in Lewickie near Białystok, Poland)
lubricant (2)	• Solution A: tetra-sodium pyrophosphate hydrated (Sigma-Aldrich) + Di-sodium dihydrogen phosphate (Sigma-Aldrich) + tetra-potassium pyrophosphate (Sigma-Aldrich) + xanthan gum (Sigma-Aldrich) in PBS (phosphate buffered saline) of pH = 7.0; • Solution B: solution of type II mucin (Sigma-Aldrich) in PBS of pH = 7.0 • Solution C: solution of type III mucin (Sigma-Aldrich) in PBS of pH = 7.0 • Solution D: human saliva
disc (3)	• Ti-6Al-4V alloy; *Ra* = 0.4 µm (ATI Germany)

tests performed under the same measurement conditions at ambient temperature. The linear wear of the pins was continuously recorded with the use of a displacement transducer**.** The surface topography of the samples and their chemical composition were examined using a Hitachi S-3000N scanning microscope (equipped with an EDS attachment for X-ray microanalysis).

After the tribological tests had been finished, the surfaces of the titanium alloy samples were tested in order to assess the adsorption level of saliva components (mainly mucins) from the contact environment. The analysis was conducted using infrared microspectroscopy.

In the present research, all the FTIR studies were done at 298 K with a Perkin Elmer Spectrum 1000 spectrometer equipped with an AutoImage IR microscope. The mid-infrared microspectroscopy measurements were carried out in the reflection mode, from square pixels 100 × 100 µm^2. The spectra were acquired in the 4000–700 cm^{-1} range, with a 2 cm^{-1} spectral resolution and 1000 scans. The background spectrum was recorded under the same conditions, prior to sample measurement, from a pure, untreated titanium surface, and it was automatically subtracted from the sample spectrum.

In order to get a comparative mucin spectrum, it was recorded from KBr pellet in the transmission mode (using an MCT detector, spectral resolution of 2 cm^{-1}, and 30 scans). All the spectra were processed using the GRAMS/AI 8.0 software (Thermo Scientific, 2006).

3. Results

During the tribological tests, the friction coefficient and the linear wear of the a-Ti alloy, depending on the lubricant, were examined.

Figures 3 and 4 illustrate the tribological test results for the a-Ti–a-Ti friction pair in the environments of artificial saliva solutions under incestigation. For all the lubricants applied, varied friction coefficient values were observed during the friction tests, (figure 3), although these values were similar in the preparations analysed. The lowest value of the friction coefficient was obtained for human saliva.

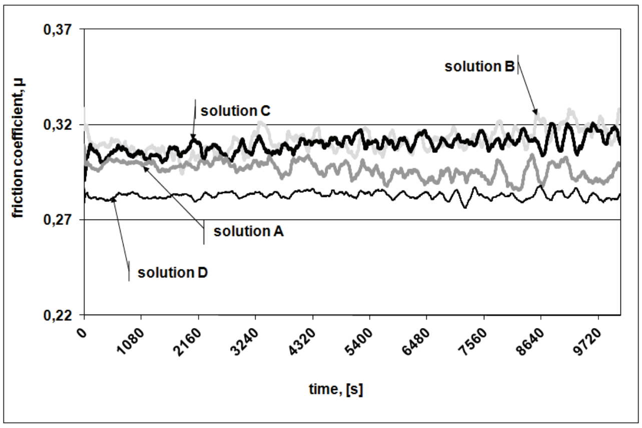

Fig. 3. The friction coefficient as a function of time for the a-Ti–a-Ti tribological pair for the environment of human saliva and for the environments of its three substitutes

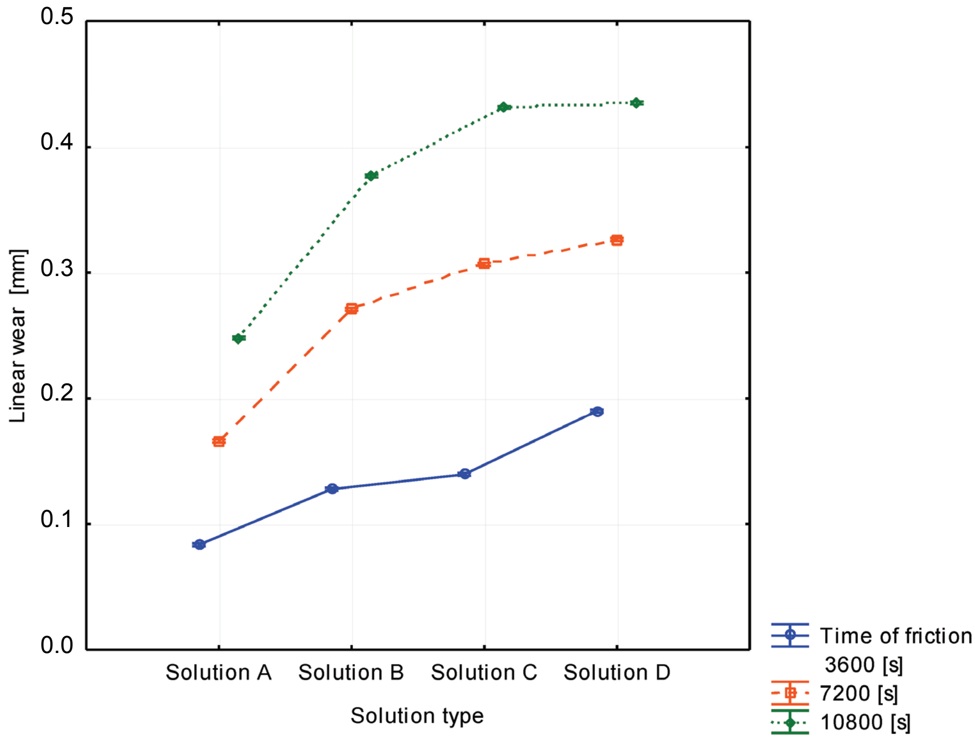

Fig. 4. Linear wear as a function of time for the a-Ti–a-Ti tribological pair for the environment of human saliva and for the environments of its three substitutes

Table 2. Linear wear of the a-Ti alloy pins (mean values from the last 100 [s] of the friction process)

Solution identification	Lubricant	Linear wear [mm]
Solution A	$Na_4P_2O_7 \times 10H_2O + H_2Na_2O_7P_2 + K_4P_2O_7$ + xanthan gum solution in PBS of pH = 7.0	0.2463
Solution B	solution of type II mucin in PBS of pH = 7.0	0.3827
Solution C	solution of type III mucin in PBS of pH = 7.0	0.4313
Solution D	human saliva	0.4351

From the diagram of the linear wear (figure 4 and table 2), it can be observed that the best lubricating substance was solution A, while the biggest wear of the titanium alloy applied was recorded for human saliva.

Loss of retention of implant-retained overdentures due to wear of the patrix or matrix of the attachment elements is a common clinical problem [21]–[23]. In [24], the authors concluded that an attachment system with ceramic matrix/gold patrix components may result in less wear than in one with titanium/gold combinations.

After the friction processes had been finished, the surface topography of the a-Ti alloy samples was evaluated with the use of a scanning microscope. Figures 5 through 8 present some selected microphotographs of the sample surfaces after the three-hour friction process in the presence of natural saliva and its artificial substitutes.

After the tribological tests in the pyrophosphate environment (figure 5) had been accomplished thin layers of secondary wear products, formed on the surface of the a-Ti sample examined during the tribological tests, were revealed.

a) b)

Fig. 5. Microphotograph of the a-Ti alloy surface after tribological tests in the pyrophosphate environment;
a) 250× magnification, b) 1000× magnification

Table 3. Results of the chemical composition analysis of the surfaces of the a-Ti samples after friction in the pyrophosphate environment

	C [%]	O [%]	Na [%]	Al [%]	P [%]	S [%]	Cl [%]	K [%]	Ca [%]	Ti [%]	V [%]	Ni [%]
1	55.06	9.39	0.54	1.18	–	0.50	0.59	0.67	–	30.92	1.15	–
2	74.71	10.70	0.43	0.70	0.16	0.35	0.31	0.55	0.35	10.79	0.47	0.47
3	10.67	–	–	3.66	0.68	–	–	–	–	81.78	3.21	–
4	5.96	–	–	4.48	–	–	–	–	–	85.59	3.97	–

a) b)

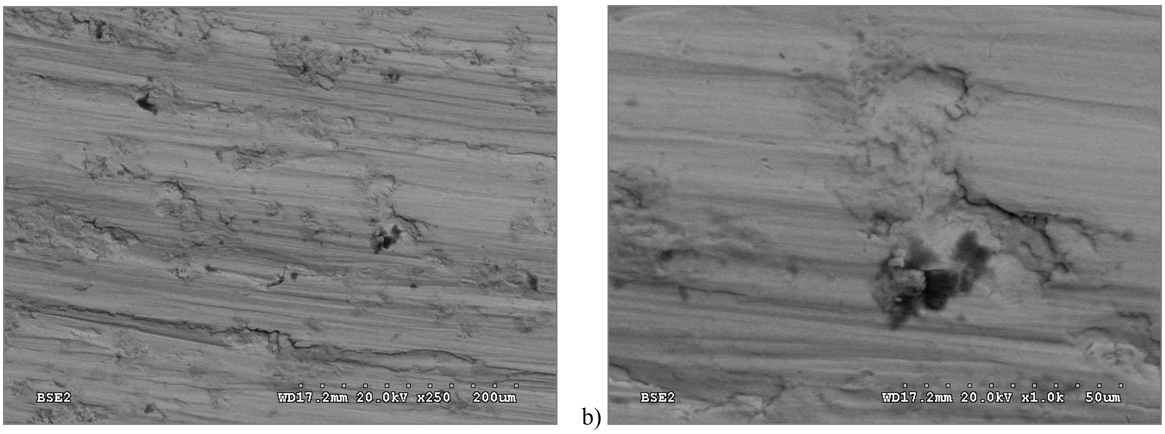

Fig. 6. Microphotograph of the a-Ti alloy surface after tribological tests in the environment of type III mucin;
a) 250× magnification, b) 1000× magnification

The EDS analysis results of the chemical composition of the a-Ti alloy surface after the friction process in the pyrophosphate environment showed an increased content of oxygen. Moreover, trace amounts of, e.g., sodium, chlorine, and potassium were found (figure 5b, table 3).

A microscopic analysis of the sample after the friction process in the environment of the solution of type III mucin in the PBS solution (figure 6) revealed destruction of the surface, with elements of abrasive wear. Areas of deeper loss of material, characteristic of adhesive wear, were visible on the surface (at a 1000× magnification).

The topography of the a-Ti alloy surface after tribological tests in the environment of type II mucin in the PBS solution (figure 7), magnified 250×, reveals particles of wear products. In the images taken at a 1000× magnification, numerous areas of material loss are visible.

In the images taken at a 250× magnification after the friction tests on the a-Ti alloy in the environment of human saliva were finished (figure 8), numerous wear products formed as a result of damaging the counterpart surfaces can be seen on the sample surface. At a higher magnification (1000×), fragments of thin films of secondary structures have been revealed.

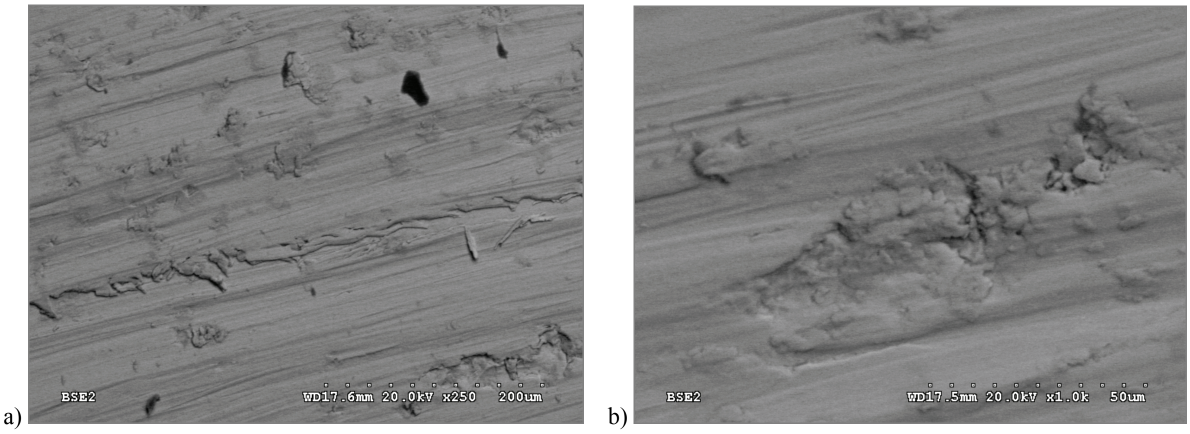

Fig. 7. Microphotograph of the a-Ti alloy surface after tribological tests in the environment of type II mucin; a) 250× magnification of, b) 1000× magnification

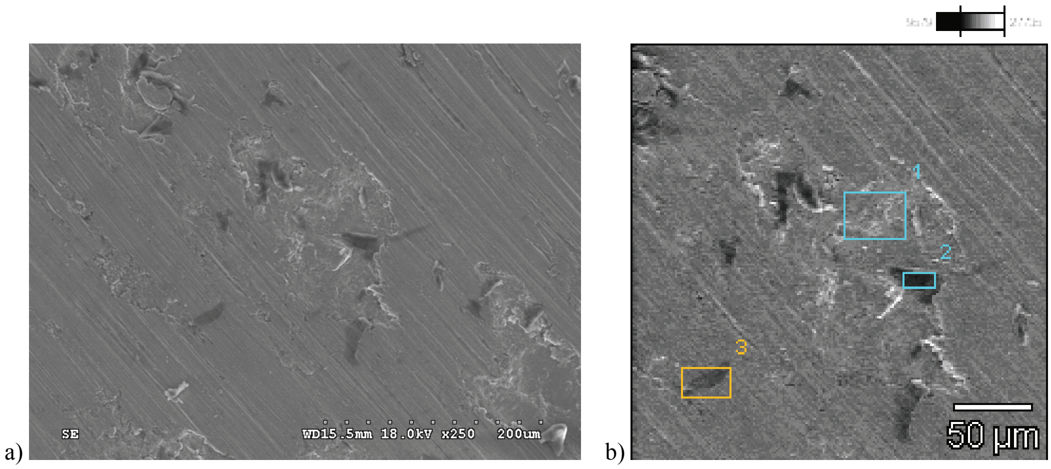

Fig. 8. Microphotograph of the a-Ti alloy surface after tribological tests in the environment of human saliva; (a) 250× magnification, (b) 1000× magnification

Table. 4. Results of tests of the a-Ti sample surface composition after friction in the environment of human saliva

	C [%]	O [%]	Na [%]	Al [%]	Cl [%]	K [%]	Ti [%]	V [%]	Ni [%]
1	9.79	–	–	3.32	–	–	83.37	3.53	–
2	53.03	12.91	0.65	1.48	0.53	0.62	29.46	1.31	–
3	30.38	7.69	–	2.55	–	–	56.66	2.69	0.02

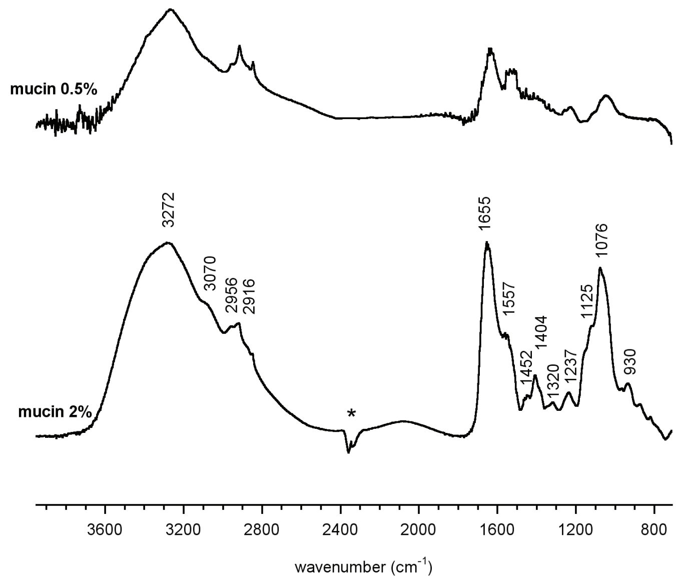

Fig. 9a. The film spectra collected from the titanium surfaces treated with mucin solutions (0.5% – top and 2% – bottom)

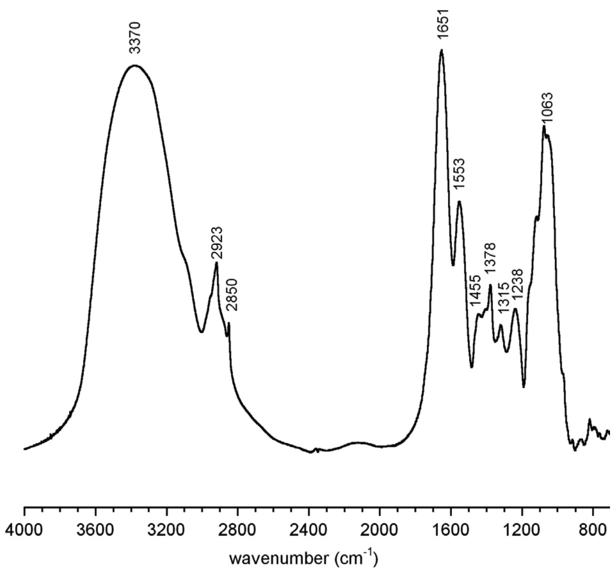

Fig. 9 b. The mucin spectrum collected from KBr pellet

Table 5. Band positions and assignments for mucin

Band (cm^{-1}) KBr disc	Band (cm^{-1}) film	Assignment	Comments
3370	3272	N–H and O–H	amide A and water
–	3070	N–H	amide B
–	2956	C–H	$–CH_3$, asymmetric stretch
2923	2916	C–H	$>CH_2$, asymmetric stretch
2850	–	C–H	$>CH_2$, symmetric stretch
1651	1655	C=O	carbonyl, stretch, amide I and water deformation
1553	1557	N–H	amide II, N-H deformation
1455	1452	C–H	$>CH_2$, deformation
1378	1404	C–O	–COO, symmetric stretch
1315	1320	C–H	δ_{CH}, ring carbohydrates
1238	1237	C–N	amide III
–	1125	C–C	pyranose ring, carbohydrate
1063	1076	C–C–O	pyranose ring, carbohydrate

A study of the chemical compositions (table 4 and figure 8b) of the products described above revealed an increased content of oxygen, and also trace amounts of sodium, chlorine, and potassium.

The last stage of the research was a FTIR analysis of the surfaces of the a-Ti samples after the tribological tests. Some selected results of this analysis are presented below.

The FTIR spectra of the thin films detected on the surfaces analysed are presented in figure 9a. Figure 9b shows the FTIR mucin spectrum recorded from KBr pellet. All the spectra contain the same bands from proteins and carbohydrates (see table 5). The intensity and resolution of the mucin 0.5% spectrum is very weak. However, there is clear evidence that the main bands correspond to mucin glycosylated proteins.

4. Discussion

Saliva fulfills many functions in the human organism. One of its more important functions is lubrication, which decreases the friction between elements of the human stomatognathic system. It is well known that the lubrication ability of saliva is associated with salivary molecules, mainly mucin, statherin or proline-rich glycoprotein [25]–[27] incorporated in salivary biofilms. Lubricating properties of saliva can be measured using different methods, i.e., friction tests. The friction coefficients described in the literature, obtained as a result of friction tests conducted in the environment of human saliva and its substitutes, are within the range of $\mu \approx 0.02–0.45$ [27].

Various material pairs (e.g., a two-metal system, metal–enamel, ceramic–enamel, dental filling–enamel) and systems (e.g., pin–disc, ball–disc) are used for the cooperating kinematic pair [28], [29] in friction tests with saliva and its substitutes. Titanium alloy can be found among the materials tested, for which, besides typical tribological tests [30], fretting-corrosion type tests are also conducted [31].

Various saliva solutions are used in tribological tests. In [32], the in vitro tribological properties of human saliva, deionized water, mucin-based artificial saliva, and carboxymethylcellulose-based saliva were examined. The wear rate was the lowest in the case of mucin-based artificial saliva. In turn, ref. [33] was a study of the influence of protein concentration on the value of the friction coefficient. The results of the study showed that the greater the protein concentration, the lower the friction coefficient for the kinematic pair.

In this work, the tribological characteristics of a kinematic pair (pin on disc) made from Ti-6Al-4V titanium alloy were evaluated in the environment of human saliva and in the environment of its substitutes. The results obtained confirm literature reports [32] that saliva solutions prepared using a mucin base (friction coefficient, $\mu \approx 0.30$) exhibit similar tribological characteristics (friction coefficient, $\mu \approx 0.28$) to human saliva. Similarly, the linear wear of titanium alloy as a function of time increased as the friction time increased, and its values were most similar for friction in a contact environment based on natural saliva and type III mucin. A novelty in this work is the use of a pyrophosphate mixture as a proposition for ingredients of artificial saliva solution. The results of our work show that the lowest friction coefficient and the lowest linear wear of the titanium alloy were obtained in this environment in comparison with the other solutions tested.

There are many methods of analysis of the chemical composition of adsorptive films on biomaterial surfaces. These include: microscopic methods (e.g., transmission electron microscopy – TEM, scanning electron microscopy – SEM, atomic force microscopy – AFM, scanning confocal laser microscopy – SCLM) [34] and methods of chemical composition analysis (e.g., mass spectrometry, infrared spectroscopy methods) [17], [34]–[36].

Following tribological studies, an analysis of the chemical composition of adsorptive films present on the surface of the titanium alloy tested was carried out by means of infrared microspectroscopy. The results of chemical analysis confirmed the presence of mucin glycosylated proteins on the surface of the titanium alloy. This is indicative of the fact that mucin adsorbs onto the surfaces of materials entering the friction area, while being decisive of the tribological characteristics of the friction system tested.

5. Conclusions

Based on the results of the research performed, the following general conclusions can be drawn:

1. The lowest linear wear value for the a-Ti alloy pins was observed for friction in the environment of pyrophosphates, whereas the highest values were obtained for the substitute based on type III mucin and for human saliva.

2. The observed friction coefficient values were similar for all the saliva substitutes applied; the comparative analysis of all the lubricating compositions shows that the lowest coefficient value was obtained in the case of human saliva.

3. In this context, it is difficult to select a composition which would be unambiguously effective, concerning its tribological properties, in the friction node under research.

4. The results of the microscopic studies reveal varied forms of tribological wear for the cooperating materials. The formation of secondary top layers containing the wear products and the lubricant components seem to be significant. Considering the character of the operation of the friction pair, it might be concluded that these layers are formed as a result of interaction between the components of the different lubricants and the surfaces of the cooperating materials.

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