Contributions Regarding the Influence of the Antibacterial Chemical Deposits on the Surface of the Oral Implant of the Ti10Zr Bio-alloy on Its Behaviour During Use

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The antioxidant and disinfectant properties of silver have been known for a long time, but explaining its antibacterial mechanism was only possible through the modern methods of investigation and analysis, such as radioactive isotopes and the electron microscopy. The effective forms of silver that determine the microbial inhibition are the silver salts, and the optimal antimicrobial effect is obtained by releasing continuously a moderate amount of silver ions. On the other hand, many studies show that the size and shape of the silver nanoparticles play a key role in their antibacterial activity. The paper presents some results of the research regarding the chemical deposition of the metallic silver with antibacterial role on the oral implant of the Ti10Zr bio-alloy. The research was conducted in the Chemistry Laboratory of the Sciences and Environment Faculty from the Lower Danube University of Galati. To establish the optimum chemical deposition conditions of the metallic silver we used several experimental regimes in different conditions of experimentation. The paper presents the experimental results obtained when introducing the implants to be covered in the Tollens's reagent following the influence of the hold time in the solution on the character of the deposition, on the dispersion, on the deposition uniformity and on particle size deposited. To cover chemically with metallic Ag we used solutions prepared with chemically pure reagents and bidistilled water under experimental regimes of chemical deposition. Electron microscopy analyses with electron scanning (SEM) and the EDX elemental analysis revealed the presence of the silver on the surface of implants, the degree of dispersion and the morphology of silver particles deposited according to the experimental regimes.

Keywords: Ti10Zr bio-alloy, chemical deposition, chemical deposition parameters, particle morphology, EDX elemental analysis

Although the antimicrobial properties of silver have been known for centuries [1,2], the mechanisms by which silver inhibits bacterial growth have begun to be understood and have been elucidated relatively recently [3,4].

At the same time it has been established that in order to have anti-microbial properties, silver must be in its ionized form (Lok et al, 2007, Rai et al, 2009). Although its non-ionized form is considered inert (Guggenbichler et al., 1999), if it is in contact with a moist environment it leads to the release of silver ions (Radheshkumar and Munstedt, 2005) [5-7].

One of the mechanisms of the antibacterial effect of silver is based on the consideration that silver atoms link to the thiol groups (-SH) of the enzymes and then determine their inactivation (Klueh et al., 2000) [6]. Also, silver ions can take part in catalytic oxidation reactions which result in the formation of disulfide bonds (RSSR). Silver does this by catalyzing the reaction between the oxygen molecules in the cells and the hydrogen atoms of the thiol groups: the water is released as a product and the two thiol groups are covalently linked to one another by a disulfide bond (Davies and Etris, 1997). Disulfide bond formation catalyzed by silver changes the enzyme cell shape and subsequently affects their function. Disulfide bond formation catalyzed by silver can lead to changes in the protein structure and to the inactivation of key enzymes such as those required for the cellular *breath* (Davies and Etris, 1997), or may even lead to cell death (Yamanaka et alia, 2005) [7,8].

Data from silver suggest that these ions denature the proteins (enzymes) of the target cell or organism by binding to reactive groups resulting in their precipitation and inactivation. Silver inactivates enzymes by reacting with the sulfhydryl groups to form silver sulfides. Silver also reacts with the amino-, carboxyl-, phosphate and imidazole-groups and diminishes the activities of lactate dehydrogenase and glutathione peroxidase [8].

Silver may be administered in several ways, but the effective forms of silver which can determine the microbial inhibition are the silver salts. Of these, silver nitrate ($AgNO_3$) was found to be the most effective in the antibacterial activity by the continuous release of a moderate amount of silver ions and by controlling the size and shape of the nanoparticles (Pal et al., 2007) [5-7]. Small nanoparticles ensure a larger amount of silver atoms which take part in the processes of cell destruction, and in terms of the particles form it was proved that the particles with different morphologies determine types of facets with a different effect on the antibacterial activity (e.g. triangular nanoparticles have more active facets compared to spherical nanoparticles, or the nanoparticles that are not

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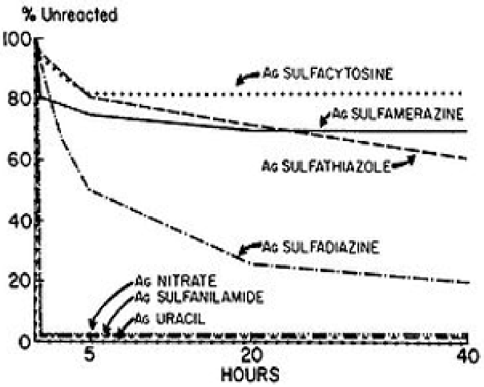


Fig.1. Percent completion of reactions of various silver compounds with human serum. Human serum was incubated with 10 µmol of each silver salt. At the intervals designated, portions were removed and centrifuged, and the clear supernatants were analyzed to determine the amount of the unreacted silver compound. The amount of compound used was taken on 100% (Fox and Modak, 1974) [4]

perfectly spherical have more active facets than the stick shaped nanoparticles (Pal et al., 2007).

Experimental part

Figure 2 illustrates the micro-structural aspects of the alloy in cast and molded plastic deformed state, respectively (extrusion) at the initial blank diameter of 5mm. Micro hardness values (HV100) attest the alloy hardening by plastic processing, without affecting the mechanical workability of the blank to achieve the final shape of the final product (dental implant) . Samples from the grade of metal alloys used in the manufacture of dental implants (Ti10Zr alloy) were embedded in epoxy resin, having previously one of the surfaces an electric conductor had been soldered, so that subsequent electrical contact can be provided (fig.3). The tests were conducted in the following simulated physiological media: artificial saliva Fusayama - Meyer (SA), Ringer's solution, Hank's solution and in 0.3M citric acid at the human body temperature (37±1°C). Experimental samples of circular shape were polished on metallographic papers with 600-1200 grit and then polished by a suspension of alumina particles of 1µm diameter to gloss mirror.

For the chemical covering with metallic silver we used solutions prepared with chemically pure reagents, bidistilled water and dental implants made from the Ti10Zr bio-alloy (fig. 2) of the self-drilling and self-screwing screw type produced at S.C. TEHNOMED Bucharest (fig. 3).

To do the research in order to establish the optimal conditions for the chemical deposition of the silver there were used several experimental regimes. The implants were placed into a receptacle containing the Tollens's reagent prepared as follows: 100 mL of 2% $AgNO_3$ solution was treated with 50 mL of 5% NaOH solution and then the resulting precipitate was dissolved by adding 50 mL of 2% NH_3 solution [5,7].

In the first regime it was added to the solution, under stirring (500rpm), 2 mL of 30% formaldehyde. The first implant was removed from the solution after 5 min and the second after 10 min of hold time. Then these were washed under running bidistilled water and then were dried in a drying cabinet at 105^{0}C for 4 h. Further on there were used experimental regimes for chemical deposition by heating the solution at different temperatures and by adding formaldehyde in droplets at intervals of 30 s.

The study of the chemical deposition in the appropriate experimental conditions was carried out by means of electron microscopy analyses with electron scanning (SEM) and the EDX elemental analysis, which revealed the presence of the metallic silver particles on the surface of the implant, the distribution, the size and the morphology of the particles deposited.

Results and discussions

In what follows we present the experimental results showing a strong correlation between the quality of the deposition and the experimental conditions for obtaining it. For a correct interpretation of the experimental results we made a comparative analysis between the implant surface without silver deposition and implant surface covered with silver nitrate in the above conditions [12].

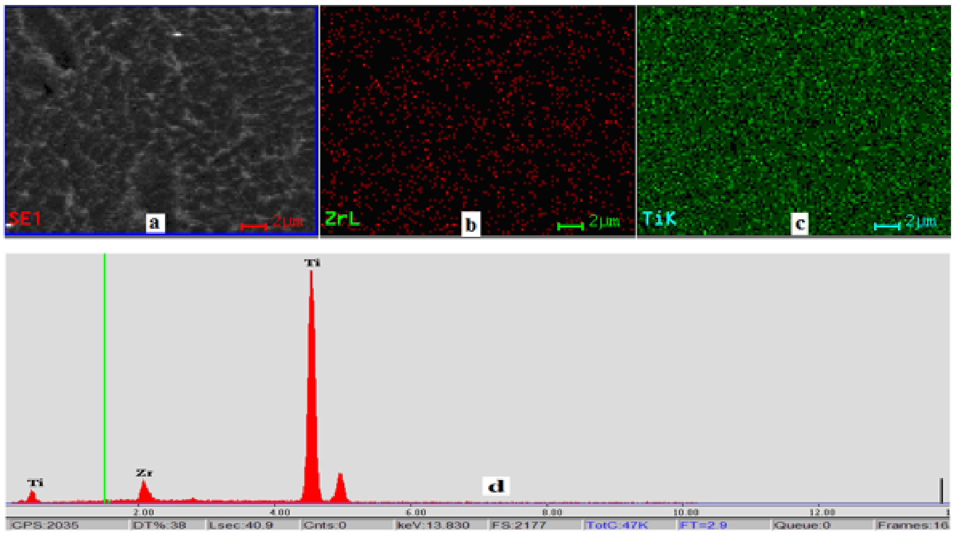


Fig.2. The SEM microscopic aspect (a), the chemical elements map (b, c) and the EDX spectres specific to the Ti10Zr bioalloy (d) [6,9-11]

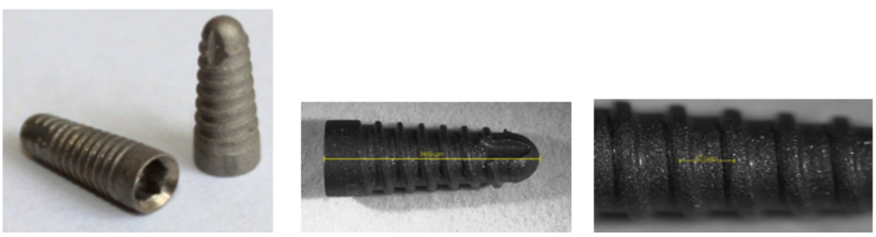


Fig.3. Ti10Zr Dental Implant of the self-drilling and self-screwing screw type [6]

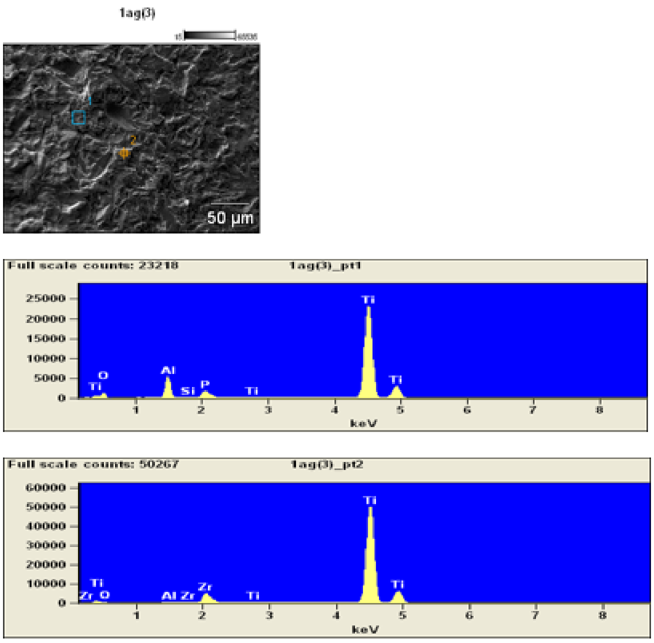
Microscopic aspects and the EDX elemental analysis of the silver chemical depostion on the dental implant surface

Corrosion is a chemical reaction between metals and environment, which causes a noticeable change in material, changing its mechanical properties and which, by its secondary metal compounds, can have negative biological effects. Considering the foregoing, the experimental research focused on the study of behaviour in specific oral environment and evaluation of the corrosion resistance of Ti10Zr alloy to assess its biocompatibility for use in dental implants. The parameter of the quantitative assessment of metals and alloys degradation by corrosion is the corrosion rate. The electrochemical method is used to estimate the amount of corroded metal by measuring the current flowing in the process. The corrosion rate is obtained with respect to the amount on the surface S and time t. By relating to the corrosion current density, the current density (I) in A/cm^2 is obtained. The experimental researches were carried out on samples from Ti10Zr alloy having the chemical composition, cut from the cast ingot with the dimensions: length 70mm and 19mm diameter. Figure 4 illustrates the Energy dispersive spectroscopy elemental analysis (EDX) performed on the implant surface without silver deposition [13, 14].

Elemental analysis results indicate chemical compositions Basic SPECIFIC mass (alloy composition, pt.2) and the oxidized (pt.1) of the samples investigated tip-implants Step-drilling oral drywall different thread. Not reveal the presence of metallic silver.

In continuation are the results of elemental analysis that highlights the presence of silver deposits in the form of particles with different morphologies and degree of dispersion, both chemical deposition system dependent (with or without stirring, a solution of immersion, with or without încălzire for maintenance undertaken and the preparation and use of reagents which are immersed the experimental implants (fig. 5, 6).

Titanium does not form toxic organometallic/protein metal complexes, or if there are, these compounds are unstable. Specialized studies show that titanium alloys are better tolerated than pure titanium, demonstrated the fact that,according to corrosion resistance, biological compatibility, price and resistance features, the alloys mostly used in medicine are “conversion” titanium-based alloys. The corrosion resistance of these alloys can be improved by alloying with molybdenum, zirconium, rhenium, niobium, chromium, manganese, resulting alloys: TiAlV, TiAlMo, TiAlCr, TiAlCrCo, etc.).The alloy most frequently used in implantology is the Ti6Al4V alloy, due to its complex favorable characteristics composed of good corrosion resistance, a low elastic modulus, good osseointegration ability and durability. However there is a number of issues related to the effects some harmful elements in the alloy composition can have, elements that are released into the tissue [8-10]. Analysis of possible reactions to prolonged contact of living tissue with the alloying elements in titanium alloys showed that the use of at least titanium alloys containing large amounts of vanadium, cobalt and nickel is not recommended. Current researches aim to develop new alloys where harmful elements are missing or replaced, for example replacement of vanadium with niobium in Ti6Al4V, Ti6Al6Nb alloys. The tests were conducted in the following simulated physiological media: artificial saliva Fusayama



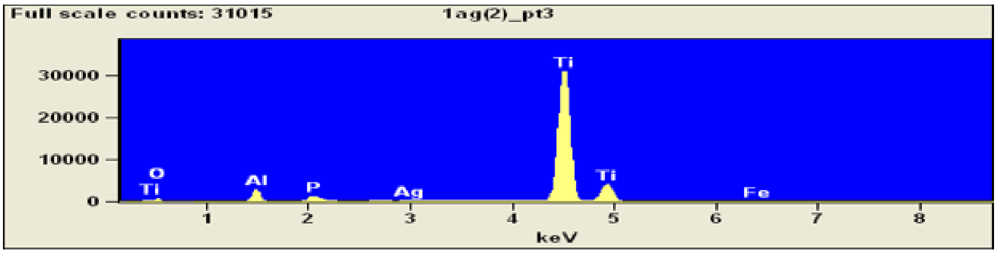
Weight %

	O-K	Al-K	Si-K	P-K	Ti-K	Zr-L
1ag(3)_pt1	20.88	7.07	0.17	1.84	70.04	
1ag(3)_pt2	0.00	0.32			91.03	8.65

Atom %

	O-K	Al-K	Si-K	P-K	Ti-K	Zr-L
1ag(3)_pt1	42.17	8.46	0.20	1.92	47.25	
1ag(3)_pt2	0.00	0.59			94.69	4.72

Fig. 4. Energy dispersive spectroscopy elemental analysis (EDX) performed on the implant surface without silver deposition



Weight %

1ag(2)_pt1	5.52	1.60	0.89	91.19		0.81
1ag(2)_pt2	9.13	1.46	0.55	88.10		0.76
1ag(2)_pt3	11.90	3.53	1.26	82.35	0.45	0.51

Fig. 5 continuated

Atom %

1ag(2)_pt1	14.72	2.53	1.22	81.21		0.32
1ag(2)_pt2	22.92	2.18	0.71	73.91		0.28
1ag(2)_pt3	28.10	4.94	1.54	64.94	0.30	0.18

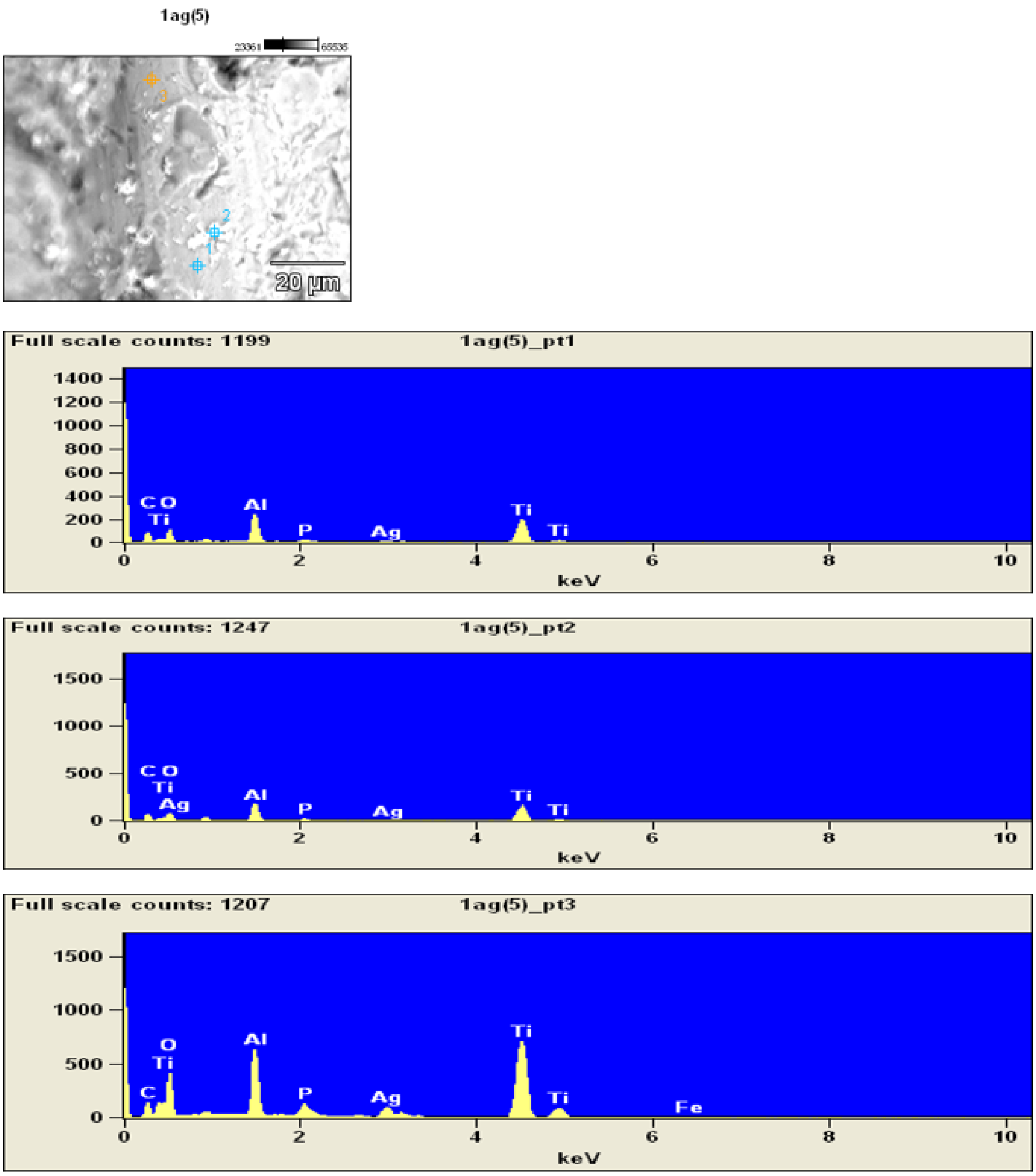


Fig.6. Energy dispersive spectroscopy elemental analysis (EDX) performed on the implant immersed in the Tollens's reagent with adding 30% formaldehyde under stirring at 500rpm, hold time 10 min

Weight %

1ag(5)_pt1	22.10	41.14	10.68	1.06	22.41		2.62
1ag(5)_pt2	24.33	40.99	9.63	1.25	21.94		1.86
1ag(5)_pt3	10.54	46.56	8.95	1.39	27.38	0.55	4.64

Atom %

1ag(5)_pt1	34.49	48.22	7.42	0.64	8.77		0.46
1ag(5)_pt2	37.10	46.92	6.54	0.74	8.39		0.32
1ag(5)_pt3	18.33	60.77	6.92	0.93	11.94	0.21	0.90

Fig. 6. continuated

- Meyer (SA), Ringer's solution, Hank's solution and in 0.3M citric acid at the human body temperature (37±1°C). Experimental samples of circular shape were polished on metallographic papers with 600-1200 grit and then polished by a suspension of alumina particles of 1μm diameter to gloss mirror.

Conclusions

The thematic studies and results of the experimental research demonstrate the possibility to treat for antimicrobial purpose the oral implants by covering their surfaces with metallic silver particles.

Silver can be administered in several ways, but its most effective formulas regarding the antimicrobial characteristics are the silver salts, of which the most used one is the silver nitrate $AgNO_3$.

The chemical deposition stage in different experimental conditions was performed comparatively by means of electron microscopy analyses with electron scanning (SEM) and the EDX elemental analysis, which showed the presence of the silver particles on the implant surfaces, their size and degree of dispersion.

The research on the chemical deposition of the metallic silver confirm the particles presence both in the deposition regimes without heating and with heating the solution to 50 and 700 C respectively and adding formaldehyde in droplets. It was found, however, that on small hold times (5 min) the silver particles are of very small size and have a low degree of dispersion. Also, the heating of the solution to a higher temperature (eg. at 700°C) causes the agglomeration of the particles of large dimensions and with a small dispersion degree which do not cover the implant surface uniformly.

The experimental researches demonstrated that when held in solution the silver particles are very small and have a small degree of dispersion as opposed to a longer holding time where the particle size increases and, also, the degree of dispersion increases.

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