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RESEARCH ON CHEMICAL DEPOSITION OF SILVER WITH ANTIBACTERIAL ROLE IN IMPLANTOLOGY

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ABSTRACT

The paper presents a synthesis of the laboratory research on the conditions of achieving chemical deposition of silver on oral implants made of Ti base alloy (bioalloy $Ti_{10}Zr$). There were used several chemical deposition regimes in which were modified deposition parameters (temperature, stirring time) for two types of implants (different screw thread geometry). Study of the influence of deposition conditions was performed through analysis at scanning electron microscope (SEM) with EDX analyzer. The results revealed the presence of silver, microdispersed particles with morphologies and degrees of dispersion dependent on the factors and technological conditions of obtaining the chemical deposition.

KEYWORDS: silver, $Ti_{10}Zr$ bioalloy, implant, chemical deposition, electron microscope SEM

1. Introduction

Properties and role of antibiotic, antioxidant and disinfectant of silver are known in the world for many centuries. Laboratory tests confirm today conclusively that silver ions have a bactericidal effect and constitute an effective disinfectant by destroying more than 600 viruses and harmful germs [1, 2]. Antibacterial efficacy of different metals has been established since 1893 and this property has been called oligodynamic effect, but later it was found that of all metals with antimicrobial properties, silver has the most effective antibacterial action and is less toxic to animal cells (Guggenbichler *et al.*, 1999).

Although known for centuries, only recently have there been understood the mechanisms through which silver antimicrobial properties inhibit the bacterial growth.

The evolution of modern ways of investigation and analysis, such as radioactive isotopes and electron microscopy has allowed explaining the antibacterial mechanism of silver (Modak and Fox, 1974; Feng *et al.*, 2000). One of these is based on the consideration that silver atoms bind to thiol groups (-SH) of enzymes and subsequently determine their inactivation. Forms of silver with S-Ag stable compounds contain in the cell membrane thiol that is involved in trans membrane energy production and transport of ions (Klueh *et al.*, 2000).

It is also believed that silver ions can take part in catalytic oxidation reactions which have as result the formation of disulfide bonds (RSSR). Silver does this by catalyzing the reaction between oxygen molecules from the cells and the hydrogen atoms of the thiol groups: water is released as a product and two thiol groups become covalently linked together by a disulfide bond (Davies and Etris, 1997).

Disulfide bond formation catalyzed by silver changes cellular enzyme form and subsequently affect their function. Disulfide bond formation catalyzed by silver can lead to changes in protein structure and inactivation of key enzymes such as those required for the "breath" of the cell (Davies and Etris, 1997), or may even lead to the death of cells (Yamanaka *et al.*, 2005).

Another mechanism which explains the antimicrobial activity of the silver has been proposed by Klueh *et al.* (2000), which shows that the Ag + enters the cell and cause its disruption.

It has been demonstrated that silver ions associate with the DNA once they enter the cell (Fox and Modak, 1974).

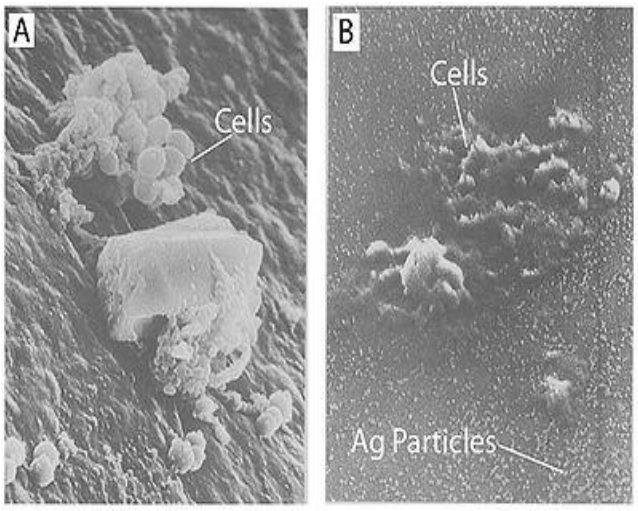


Fig. 1. *Treatment of cells with Ag^+results in DNA condensation, cell wall damage, and silver granule formation. (A) E. coli and (B) S. aureuscells with and without Ag^+treatment were observed with transmission electron microscopy (Fenget* et al.*, 2000)*

In order to have anti-microbial properties, silver has to be in its ionized shape (Lok *et al.*, 2007; Rai *et al.*, 2009). Silver in its non-ionised form is inert (Guggenbichler *et al.*, 1999), but in contact with a moist environment leads to the release of silver ions (Radheshkumar and Munstedt, 2005). Therefore, all forms of silver or silver as compounds with anti-microbial properties are in one way or another the source of silver ions (Ag +); these silver ions can be incorporated and released slowly. Feng *et al.* (2000) conducted a study to see the effects of ions of silver per gram-positive and gram-negative bacteria, such as Staphylococcus aureus and Escherichia coli. They treated cells with $AgNO_3$ which is a source of Ag + in aqueous medium, and they observed structural and morphological effects of these ions of silver on cells. Cells were exposed to $AgNO_3$ for 4-12 hours before being prepared for microscopy (TEM). There was some deterioration of the cell wall and outer dense particles, and in some cases within the cell (Figure 3). Dense particles which were formed inside and outside the cell were extracted and subjected to the X-ray microanalysis for the determination of their composition. It was found that the particles were made of silver and sulfur. This finding supports the idea that silver inactivate binding proteins to compounds that contain sulfur (Klueh *et al.*, 2000). It was also observed that when treated with Ag +, E. coli, a gram-negative bacterium causes more sustained structural damage than gram-positive S. aureus (Feng *et al.*, 2000). It was also shown that treating cells with silver leads to dehydration and contraction of the cell (Guggenbichler *et al.*, 1999). TEM images from Feng at. al. (2000) show that cells which underwent extensive damage, finally will end with the deterioration of the cell membrane. Cells membrane damage leads to the elimination of the cytoplasm, such as dehydrated and wrinkled cells are shown in the SEM images from Guggenbichler *et al.* (1999). Silver can be managed in different ways. The various effective forms of silver that contribute to the inhibition of microbial are silver salts. One of these is the silver nitrate ($AgNO_3$) and is effective because it can provide a large amount of silver ions at once. It turned out that prolonged antimicrobial activity of silver is best achieved through the continuous release of moderate amounts of silver ions. In addition, the size and shape of the nanoparticles play also an important role in the antibacterial activity (Pal *et al.*, 2007). Smaller nanoparticles require less time for penetrating through the cell membrane and cell wall in relation to larger nanoparticles that have a higher surface-volume ratio (Martinez-Castanon *et al.*, 2008). This means that per mass unit, smaller nanoparticles provide more silver atoms in contact with the solution than do large nanoparticles, meaning there are more silver atoms able to take part in the processes of cell destruction. Regarding the form of nanoparticles with a role in the antibacterial activity, Pal *et al.* (2007) had experienced three types of silver nanoparticles (spherical in the form of rods and triangular tested on E. coli.). It was found that the order in which the antibacterial effect manifested was the triangular, spherical shape and the rod-shaped form. This order of antibacterial activity is explained by the different types of veneers on nanoparticles. Triangular nanoparticles have more active facets (electrons dense facets) than spherical nanoparticles. Spherical nanoparticles, which were not perfectly spherical, have more active facets than the nanoparticles in the form of rods (Pal *et al.*, 2007).

2. Experimental conditions

For the chemical coating with metallic silver there were used solutions prepared with pure chemical reagents, double distilled water and two types of dental implants made from $Ti_{10}Zr$ alloy (developed at SC TEHNOMED Bucharest). The research was conducted in the Laboratory of Chemistry of the Faculty of Science and Environment of "Dunarea de Jos" University of Galati.

Chemical staining was performed as follows: 100 ml of a 2% $AgNO_3$ was treated with 50 ml of 5% NaOH, and then the resulting precipitate was dissolved by adding 50 ml 2% NH_3 solution. The implants (pins) were inserted in a 250 mL container in Tollens reagent obtained according to the recipe above. Also, a magnetic stirrer was inserted to achieve the homogenization of the system. Under stirring at 500 rpm, 2 ml of formaldehyde 30% were added.

The first pivot was extracted from the solution with tweezers after 5 minutes and the second pivot after 10 minutes. The two pins were rinsed in double distilled water and placed for drying in an oven at 105 ºC for 4 hours.

2.1. Method Principle Chemical reactions in metallic Ag deposition:

$(Ag^+ + NO^{3-}) + (Na^+ + HO^-) = Ag(OH)$ (white pp.)$\downarrow + (Na^+ + NO^{3-})$ (1)

$2\,AgOH \rightarrow Ag_2O$ (black pp.)$\downarrow + H_2O$ (2)

$Ag_2O + NH_3 + H_2O \rightarrow ([Ag(NH_3)_2] + HO^-)$ (complexcolorless combination) (3)

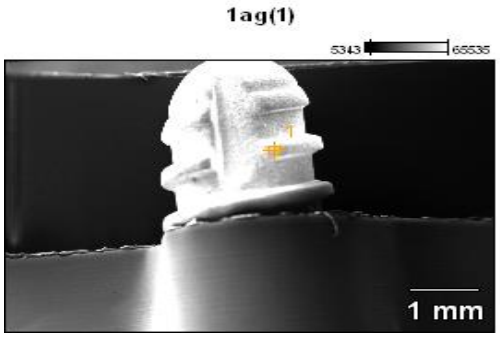
$H-C(=O)-H + 2([Ag(NH_3)_2] + HO^-) \rightarrow 2\,Ag + H\text{-}C(=O)OH + 4NH_3$ (4)

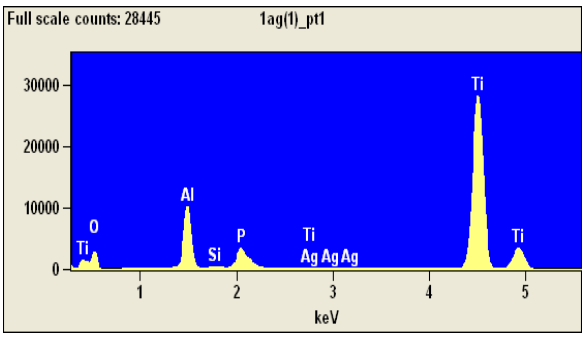
The second regime of chemical deposition using the same pivots, however, the solution heated at a temperature of 50 ºC with hold for 5 min and dripping formaldehyde from 30 to 30 seconds. The regime was repeated in the same conditions for the temperature of 70 ºC. Experimental samples were analyzed by the electron scanning electronic microscopy (SEM) with the EDX analyzer. In the next paragraphs the results obtained are presented, which revealed the presence of silver, microdispersed particles having morphologies and degrees of dispersion according to the factors and technological conditions of obtaining the chemical deposition.

3. Experimental results

The SEM microstructural aspects and EDX analysis of pivots (dental implants made at SC Tehnomed Bucuresti SA from $Ti_{10}Zr$ bioalloy).

- Regime 1 (5 min. maintaining in solution with stirring, without heating)





Weight %

	C-K	O-K	Al-K	Si-K	P-K	Ti-K	Ag-L
1ag(1)_pt1	2.78	26.15	8.68	0.16	2.34	59.47	0.42

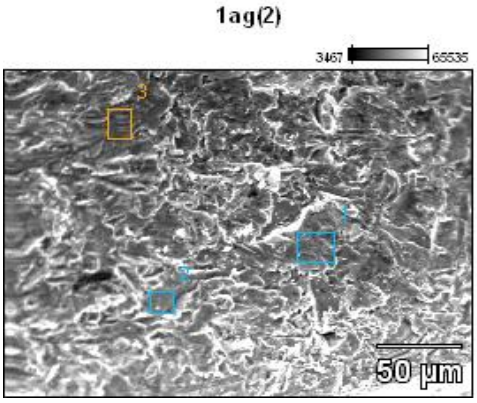
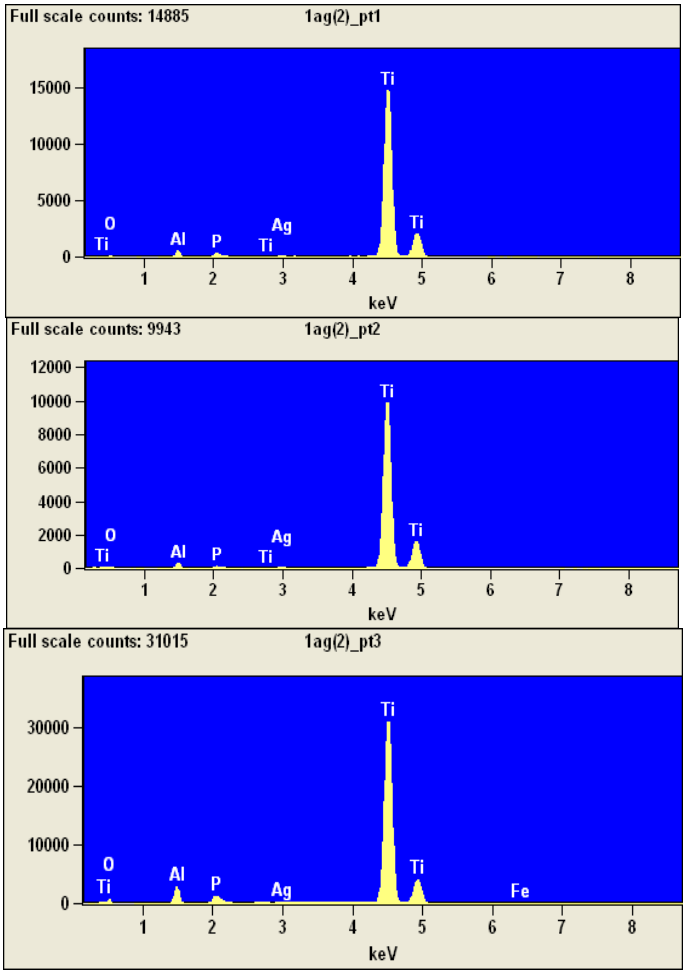


Image Name: 1ag(2)

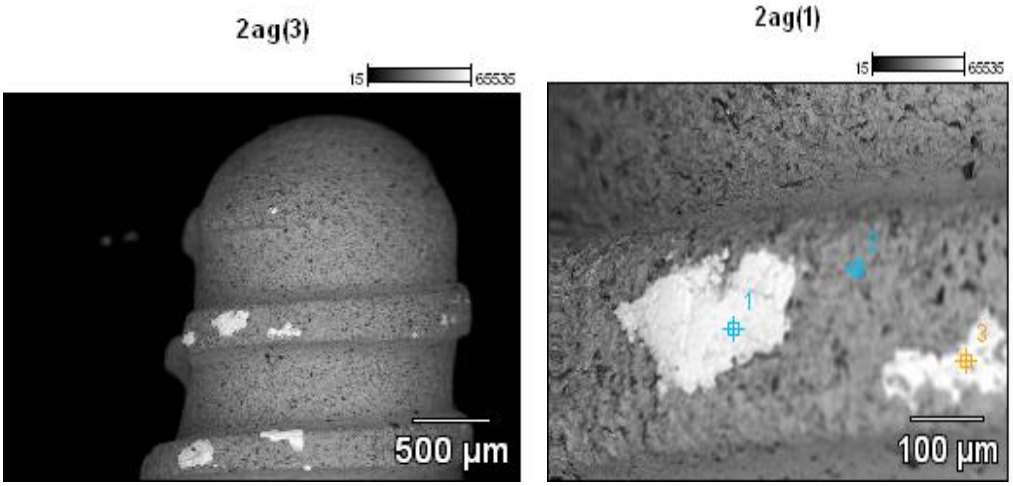
Accelerating Voltage: 20.0 kV

Magnification: 1128



	O-K	Al-K	P-K	Ti-K	Fe-K	Ag-L
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

- Regime 2 (10 min. maintaining in solution with stirring and heating at 50 ºC)



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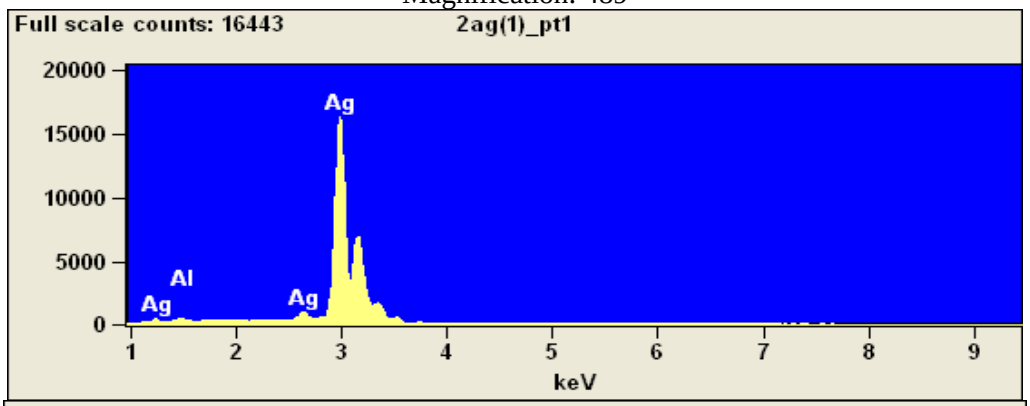

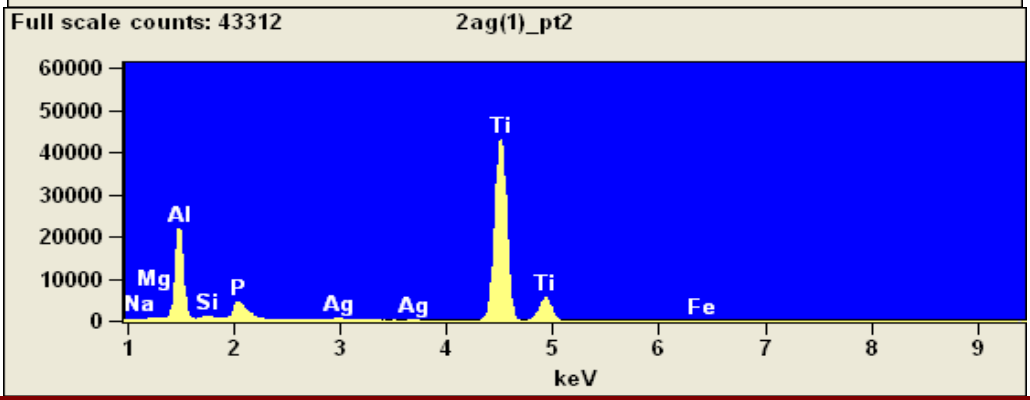

	O-K	Na-K	Mg-K	Al-K	Si-K	P-K	Ti-K	Fe-K	Ag-L
2ag(1)_pt1				0.49					99.51
2ag(1)_pt2	**40.40**	**0.04**	**0.11**	**10.19**	**0.23**	**1.66**	**46.77**	**0.28**	**0.33**

4. Conclusions

The experimental results on the chemical deposition of metallic silver on the implant built form $Ti_{10}Zr$ alloy confirm the presence of particles both in regimes of deposition without heating but also in those which were made by heating the solution at 50 ºC respectively 70 ºC and added formaldehyde drop by drop. It was noted, however, that at shorter maintenance periods (5 minutes) the silver particles have small dimensions and a low degree of dispersion. Also, heating the solution at 70 ºC does not change size, shape or degree of dispersion of metallic silver particles deposited at 50 ºC solution temperature. Electronic microscopy analysis with electron scanning (SEM) and energy dispersive spectroscopic analysis (EDX) revealed the presence of silver particles microdispersed having

morphologies and degrees of dispersion dependent on the technology of obtaining the chemical deposition and enables formulating a conclusion regarding the optimum regime. Particles with a high compactness and dispersion degree are obtained by heating the solution at temperatures of about 50 ºC, maintaining about 10 minutes with shaking and additions of formaldehyde drop by drop.

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