THE EFFECT OF ADDING TiO_2 NANOPARTICLES ON DENTAL AMALGAM PROPERTIES

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Abstract:*In recent years, there have been many attempts to improve the properties of dental amalgam. The aim of the present investigation was fabrication and characterization of dental amalgams containing TiO_2 nanoparticles and evaluation of their compressive strength, antibacterial and corrosion behavior. In this experimental research, TiO_2 nanoparticles (TiO_2 NPs) were added to reference amalgam alloy powder and then, dental amalgam was prepared. In order to investigate the effect of TiO_2 NPs on properties of dental amalgam, 0, 0.5, 1, 2 and 3 wt. % of TiO_2 NPs were added to amalgam alloy powder and the prepared composite powder was triturated by a given percent of mercury. X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy-Dispersive Spectroscopy (EDS) techniques were used to characterize the prepared specimens. Potentiodynamic polarization corrosion tests were performed in the Normal Saline (0.9 wt. % NaCl) Solutions as electrolytes at 37°C. The results showed that the corrosion behavior of the dental amalgam with 0.5 or 1 wt. % TiO_2 NPs is similar to the corrosion behavior of the reference amalgam, while with increasing the weight percent of TiO_2 NPs, the corrosion rate increases. Also, the results of this investigation indicated that adding TiO_2 NPs in amounts of up to 1 wt. % to amalgam alloy powder improve compressive strength of dental amalgam and has no destructive influence on its corrosion behavior. As well as, according to antibacterial results, TiO_2 NPs can increase the biocompatibility and antibacterial activity of dental amalgam. The results of present study suggest that amalgam/ TiO_2 NPs nanocomposite with 1% of TiO_2 NPs could be regarded as a biocompatible and bioactive dental material that provide better characters for dental applications.*

Keywords: *Dental amalgam, TiO2 nanoparticles, corrosion behavior, antibacterial properties, compressive strength.*

1. INTRODUCTION

Dental amalgam is a metallic alloy formed by the reaction of mercury with a powder alloy containing silver (40–70%), tin (15–30%) and copper (10–30%), and sometimes also a small percentage of zinc [1]. It has been used as a restorative material for the replacement of the decayed tooth structure for more than 150 years [1-3]. Hence modified and improvement of its properties were following [4-10].

From a biocompatibility viewpoint, the corrosion of an alloy indicates that some of the elements are available to affect the tissues surrounding it. Corrosion is a chemical property of a alloy that has consequences for other its properties, for instance esthetics, strength, and biocompatibility[11] Corrosion of amalgam has usually been evaluated by electrochemical polarization tests [2,4,12].

Up to now, there have been many attempts to improve properties of dental amalgam that these comprise treatment on amalgam alloy powder [5, 13] or addition some materials such as In, Au, Pa and organic materials [6-8,10,14]. One of the latest additive materials is Palladium [8,15]. Colon et al. were evaluated influence of palladium addition and particles morphology on corrosion behavior of dental amalgam. They showed adding 0.5 wt% Palladium in a high copper amalgam powder improves the corrosion behavior of amalgam up to a period of 10 years [12]. Another new additive materials was used, was Ag-Cu nanopowder. Chung et al. were

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investigated morphology and electrochemical behavior of Ag–Cu nanoparticle-doped amalgams. They indicated that the significant increase in the corrosion resistance of Ag–Cu nanoparticle-doped amalgams indicate the potential of nanoparticle amalgam development [4].

TiO_2 have good antibacterial properties and is not toxic [16]. so it was selected as an additive to dental amalgam. Moreover, the antibacterial properties depend on the surface a materials and increased with decresing size of particles so TiO_2 nanoparticles (TiO_2 NPs) have more antibacterials properties than micropowder of titania [16-18].

The aim of present research was fabrication and characterization of amalgam/TiO_2 NPs nanocomposite and evaluation of its corrosion behavior, antibacterial activity and compressive strength. Therefore, in this study TiO_2 NPs with varying amounts were added to amalgam alloy and mentioned properties of the amalgam/ TiO_2 NPs nanocomposites was compared to the reference dental amalgam.

2. EXPERIMENTAL PROCEDURE

Characterizations of used dental amalgam and TiO_2 nanoparticles listed in Table 1. The mercury to alloy powder ratio was 42.5%. The size of its particles was measured by Image Tools analysis sotware and was <60 µm. The amounts of 0.5, 1, 2 and 3 weight percent of nanoparticles of TiO_2 NPs were added to amalgam alloy powder and were prepared Am-0, Am-0.5, Am-1, Am-2 and Am-3 nanocomposites, respectivity. Weighting was done by a digital balance with 0.0001g accuracy. Also, a sample was fabricated without additive as reference amalgam. Then, this mixed powder was triturated by mercury in the time and frequency given by producer instruction via a digital capsule amalgamator (Farazmehr corporation, Iran). The amalgam mix was condensed into a Plexiglass mold with 10 mm ×10 mm ×3mm using a condenser (Φ3 mm, Aesculap, Germany). One hour after condensing, specimens were taken out from the mold and were hold for 24 hours in the room temperature. Then each sample was placed in a sealed plastic container in an incubator at 37 °C for one day. After that, the surfaces of specimens were polished by 2400 and 4000-grit SiC papers with water coolant. The samples were washed by distilled water and were cleaned by acetone to evaluation corrosion behavior.

Potentiodynamic polarization tests were performed in a three-electrode cell by an electronic potentiostat/galvanosta (PARSTATE, 2273, USA) interfaced with a computer and a recorder. The reference electrode was a saturated calomel electrode (SCE) and Pt used as counter electrode. Also, the Normal Saline (0.9% NaCl) solution was utilized as electrolyte at 37±1°C. After reaching to a steady open-circuit potential (OCP) Potentiodynamic polarization test was started from 250 mV more cathodic than the OCP with scan rate of $0.5mVs^{-1}$. Three replicate tests of each group of specimens were done. The anodic and cathodic polarization curves were obtained for each specimen. Tafel extrapolation

Table 1. Starting material Specifications

Starting materials	Tradename	Particle size	Element compositions	Manufacturer
Dental amalgam	Tytine	<60 µm	59wt% Ag, 28 wt% Sn and 13 wt%Cu	Kerr, USA
TiO_2 NPs	Evonike	21 nm	TiO_2	Germany
TiO_2	Merck	1-5 µm	TiO_2	Germany

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polarization methods determined corrosion potential and corrosion current densities. The mean value and standard deviations of the results were calculated and reported.

In order to preparing samples for compressive strength was used the mentioned procedure but the homogeneous amalgamated amalgam was put on the hole of a stainless steel cylindrical mold with the diameter and height of 4.0 + 0.1 mm and 8.0+ 0.1 mm, respectively. The measurement of compressive strength was performed by a Hounsfield (H25 KS, England) machine which connected a computer system and applied a pressure in the direction of the axis of the test with the speed of 0.5mm per minute. Ultimate compressive strength (UCS) of samples was calculated from the formula $UCS=4F/\pi d^2$, where F is maximum applied load (N) and d the cylindrical specimen diameter (mm). Five specimens were prepared for measuring each group. These samples were holed in an incubator at 37 ± 1°C for 24 hours. Afterwards, the compressive strength test was done.

In order to evaluation of antibacterial activity of reference dental amalgam and amalgam/ TiO_2 NPs nanocomposite, has been used spectroscopy procedure. The specimens were prepared by mixing alloy powder composite with mercury as the mentioned procedure. The triturated amalgams were condensed by hand in a 10 x 6 x 2 mm Plexiglas mold. The antibacterial activity of the prepared samples was studied against Streptococcus mutans bacteria (ATCC: 35668, was obtained from Iranian Research Organization for Science and Technology, Tehran, Iran). Streptococcus mutans is one of the bacteria that it causes caries and has been found to be the initiator of most dental caries.

An overnight culture of Streptococcus mutans was inoculated into fresh Muller Hinton broth medium medium. Optical density (OD) of the bacterial suspension was adjusted to 0.6 for Streptococcus mutans by means of a visible spectrophotometer (Beckman, M24). A 5-mL quantity of this bacterial suspension was added several Pyrex test tubes containing one of the amalgam samples. Controls (Co) were without amalgam. (All tubes were inoculated from the same generation of the bacterial strain). The samples were tested 24 h after trituration since according to other works, the cytotoxicity of amalgams decreased considerably from the one-hour to the 24 h ageing period but afterward remained relatively constant [19]. A tube containing 5 mL of bacterial suspension without amalgam was considered as Controls. All tubes were incubated aerobically at 37°C for 24 h, and optical density measurements at 640 nm were done at 0, 4, and 24 h. From the values obtained at 24 h, the average number and standard deviation were evaluated. These results were analyzed by analysis of variance and t test for multiple comparisons between means at the 5% level. The purity of the cultures was monitored throughout the experiment by examination of Gram stains of the cultures and by visual identification [19].

X-ray Diffraction (XRD) technique (Philips Xpert, Philips, Holland) was used to evalusion phases in the reference amalgam and the new amalgam with different amounts additive and scanning was done from 10° to 80° (2θ) in a step size of 0.02°. Scanning Electron Microscopy (SEM) and Energy diffraction Spectroscopy. X-ray Diffraction (EDS; Xpert, Philips, Holland) with Cu target (λ= 1.542A°) was done from 10° to 80°. The microstructure, morphology and elemental composition of the prepared samples were characterized using scanning electron microscope (SEM) equipped with an X-ray energy dispersive spectrometer (EDS, Seron, AIS 2100, korea).

3. RESULTS AND DISCUSSION

Figure 1. indicate X-ray patterns of amalgam alloy powder and TiO_2 NPs as starting materials. The phase evalution showed that amalgam alloy powder has only Ag_3Sn and Cu_3Sn as well as, TiO_2 NPs contain two phases of TiO_2 (Anatase and Rutile). The XRD results of reference dental amalgam and dental amalgams with different percent of TiO_2 NPs are shown in Fig. 2.a. It confirmed the presence of Cu_6Sn_5 (ε), Ag_3Sn (γ), Ag_2Hg_3 (γ1) phases and amount of Ag_4Sn. Indeed, after mixing with mercury, there would be unreacted phase of Ag_3Sn, new phase of Ag_2Hg_3 and most of Cu_3Sn convert to Cu_5Sn_6

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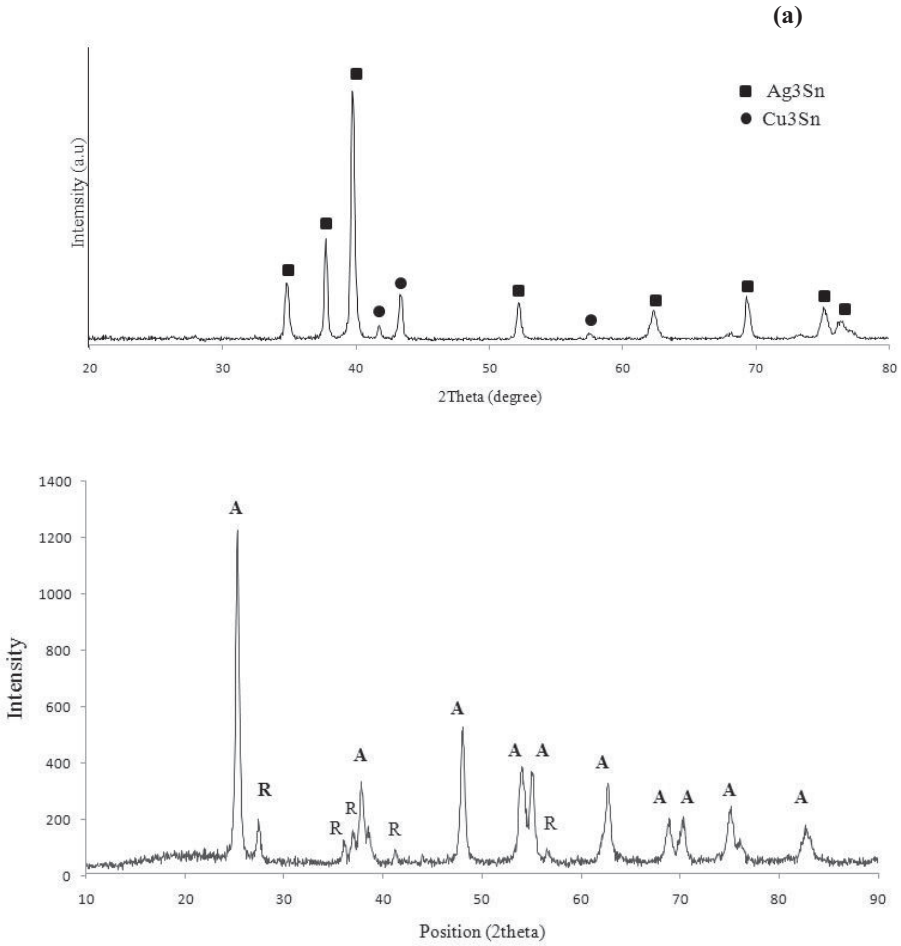

Fig. 1. XRD pattern of a) amalgam alloy powder and b) TiO2 NPs.

[1,4] (Fig .2b). This is evidence that there is not Ag-Hg phase in the reference amalgam and other samples and it was in close agreement with the findings of previous studies [1, 3]. By considering of the XRD results of dental amalgams with different percent of TiO_2 NPs no significant differences were identified by adding TiO_2 NPs and they comprise the phases present in the reference amalgam. In addition to, it seems that main peaks of TiO_2 NPs had been placed near to peaks of amalgam. Thus, identifing of TiO_2 NPs is difficult. So, for better evaluation, we prepared a sample with 6wt% micropowder of TiO_2 (Fig 2.b). It should be noted that the maximum amount of TiO_2 NPs as an additive to dental amalgam was 3wt% but it had been 6 wt. % for TiO_2 micropowder. It is due to the very small particles and high specific area of TiO_2. NPs, so that the amalgamation process faces disturbance in higher level than 3wt. % TiO_2. NPs. This state could make when TiO_2 micropowder add to dental amalgam in higher amounts of 6 wt. %. As can be seen important peaks of TiO_2 had overlapped with dental amalgam phases and there is one peak in 2θ= 48.2, just for TiO_2. Subsequently, it can be said that TiO_2 NPs were distributed homogenously or its amounts were not enough to detecting by XRD analysis. Figure 3.a, display SEM micrograph of amalgam alloy powder with spherical morphology and particle size< 60 µm. The back-scattered electron scanning images of reference amalgam is shown in Figure 3.b and different regions with different phases were

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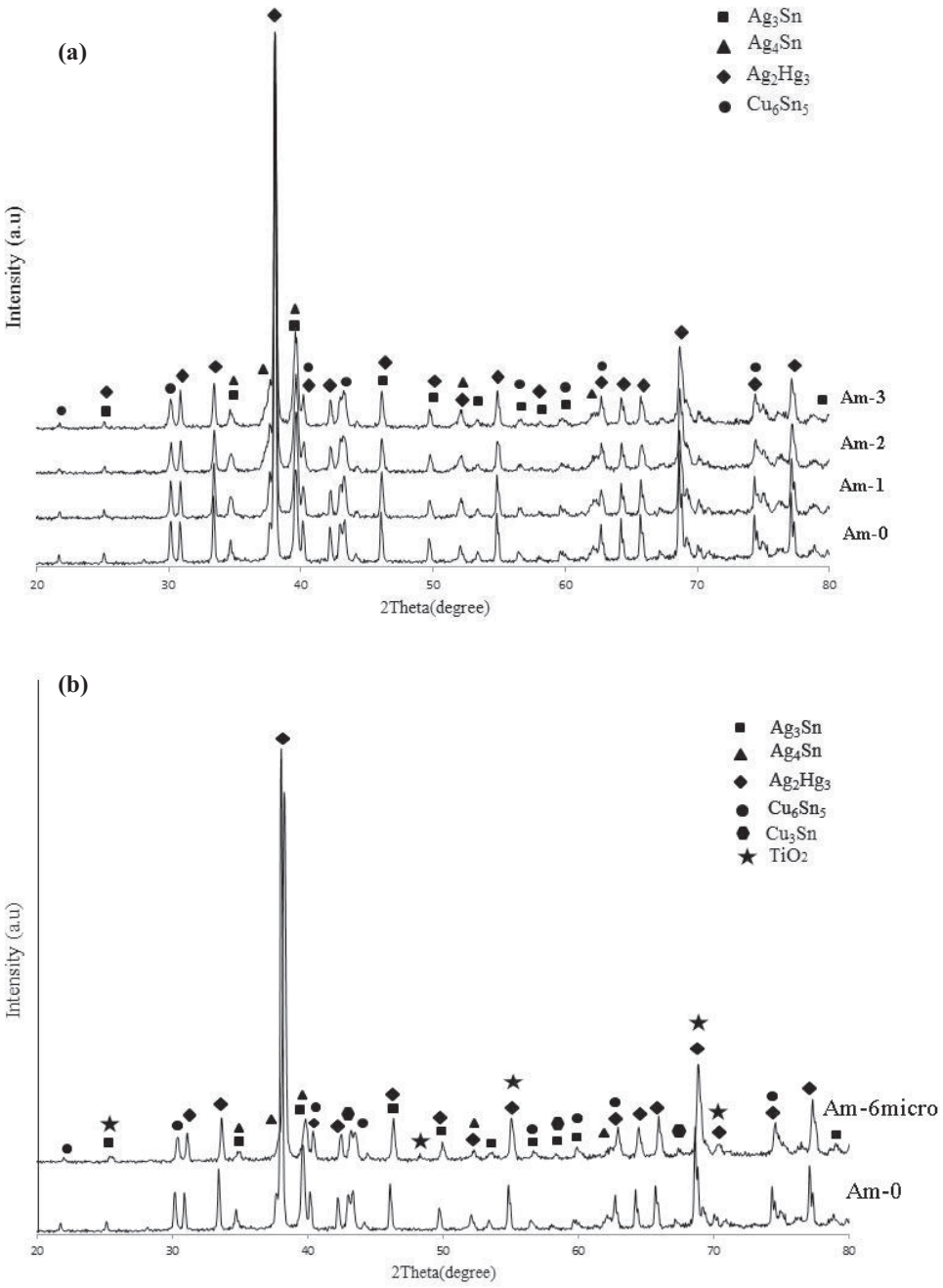

Fig. 2. XRD results of reference dental amalgam with (a) dental amalgams containing 1,2and 3 wt% of TiO_2 NPs and (b) dental amalgam with 6wt% micro TiO_2.

marked. In Figure 4.b dental amalgams with different percent of TiO_2 NPs are indicated. As it can be seen both of the reference amalgam and amalgam/TiO_2 NPs nanocomposites contain Ag_3Sn (γ), Ag_2Hg_3 (γ1) and Cu_5Sn_6 (ή). Also, it has been achived a homogeneous distribution of phases and did not have any change in phase distribution by adding TiO_2 NPs.

The EDS results of dental amalgam with 1.5 weight percent TiO_2 NPs are indicated in Fig. 5. Also, the EDS spectrum of this area was shown (Fig. 5b). In order to better assess, three regions

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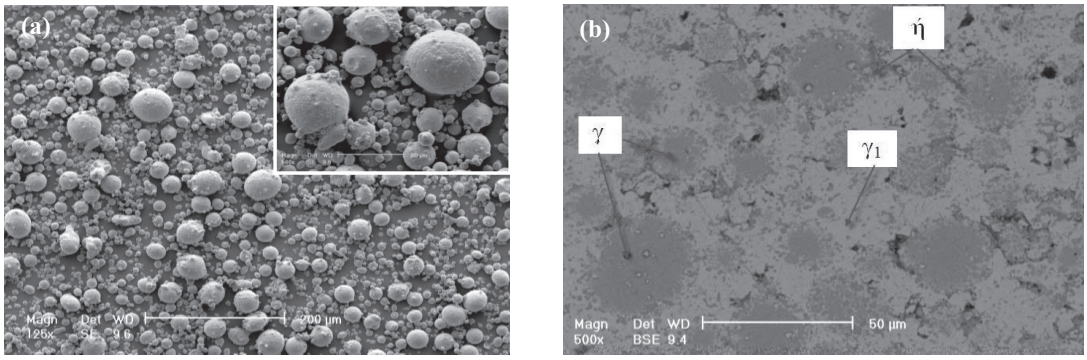

Fig. 3. SEM micrograph of reference dental amalgam: a) amalgam alloy powder, b) reference dental amalgam.

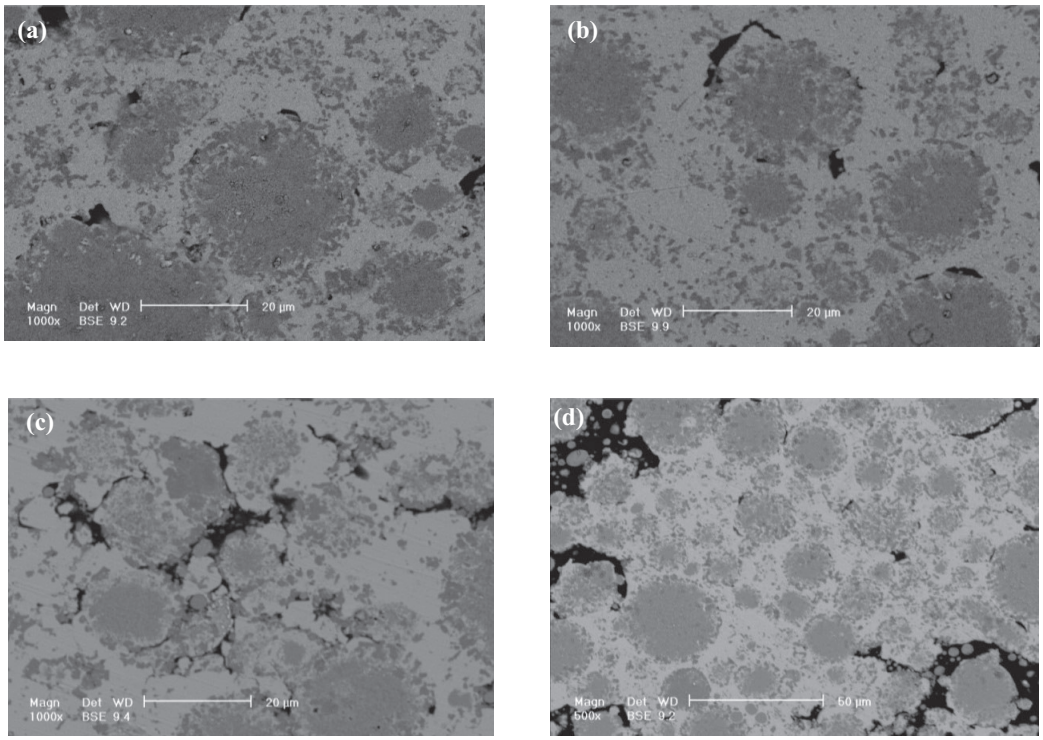

Fig. 4. SEM micrographs of amalgam/ TiO_2 NPs nanocomposites (a) 0.5 wt. % , (b) 1 wt. %, (c) 2 wt. % and (d) 3 wt. % of TiO_2 NPs.

were selected: (1) First region contains all of present phases in the dental amalgam; (2) second region contains mainly γ_1 and (3) Third region largely contains γ_1 and ή. The results of total area was shown 45.63 wt% Ag, 20.03 wt% Sn, 6.05 wt% Cu, 26.25wt% Hg and 1.43 wt. % Ti. So, present of 1.5 wt% Ti in the sample is logical. The second area has more γ phase, thus it is expectable to more Ag and Sn in the EDS results (21.5 wt. % Sn and almost 65.9 wt. % Ag). Accordingly amount of Ti was a little (about 0.4 wt. %). The matrix of a dental amalgam mostly has γ_1, so it would be more mercury in the EDS analysis of this area. Also, there was almost 1.6

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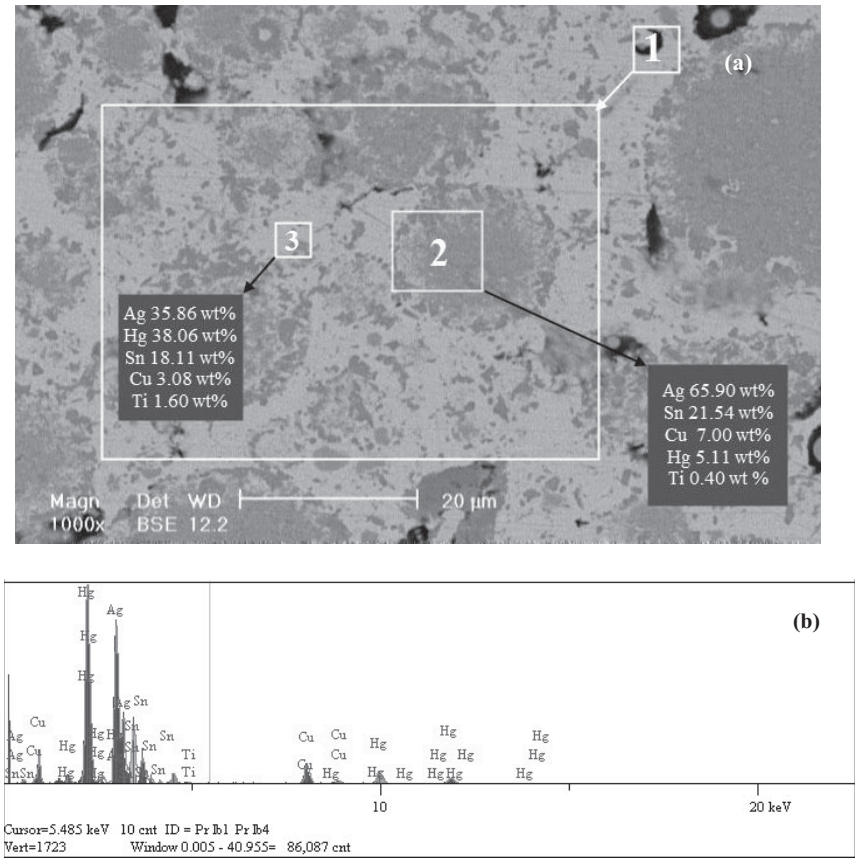

Fig. 5. EDS results of dental amalgam with 1.5 wt% TiO_2 NPs; (a) SEM micrograph and EDS results for 1 and 2 region and (b) spectrum of EDS analysis for the first region.

wt. % Titanium and 3.2 wt. % Oxygen in the matrix, hence, it can be concluded that TiO_2 NPs was significantly existed in the matrix.

Corrosion behavior of dental amalgams with different percent of TiO_2 NPs was evaluated by the electrochemical corrosion test in normal saline solution. The polarization curves of reference amalgam and dental amalgams with different percent of TiO_2 NPs were shown in Fig. 6. The related corrosion current densities and corrosion potentials were listed in Table 2. The results showed statistically significant differences between the mean corrosion current densities values of 3 different groups of the samples ($P<0.05$). There is not a significant difference between reference dental amalgam and amalgam/ TiO_2 NPs consisting of 0.5 wt. % or 1 wt. % TiO_2 NPs, but by increasing the weight percent of TiO_2 NPs, the corrosion current density increase and therefore corrosion rate is higher. This occurrence can be related to the microstructural changes of nanocomposite that appear in higher level of TiO_2 NPs. The high amounts of TiO_2 NPs makes large porosities in near the unreacted alloy particles (as seen in figure 3. c and d) and these weaken properties of dental amalgam; because the porosity have the lowest corrosion resistance and led to decrease compressive strength [4,20]. So, presence of large porosities in surrounding to alloy particles is responsible of decline in corrosion resistance.

Table 3 showed the results of compressive strength tests with increasing added TiO_2 NPs amount to amalgam. The compressive strength firstly increases (for the sample with 0.5 and 1 wt. % TiO_2 NPs) and then decreases (for the samples with 2 and 3 wt. % TiO_2 NPs). The enhancement of compressive strength of dental amalgams has been reported in previous studies. For instance, the researcher presented that dental

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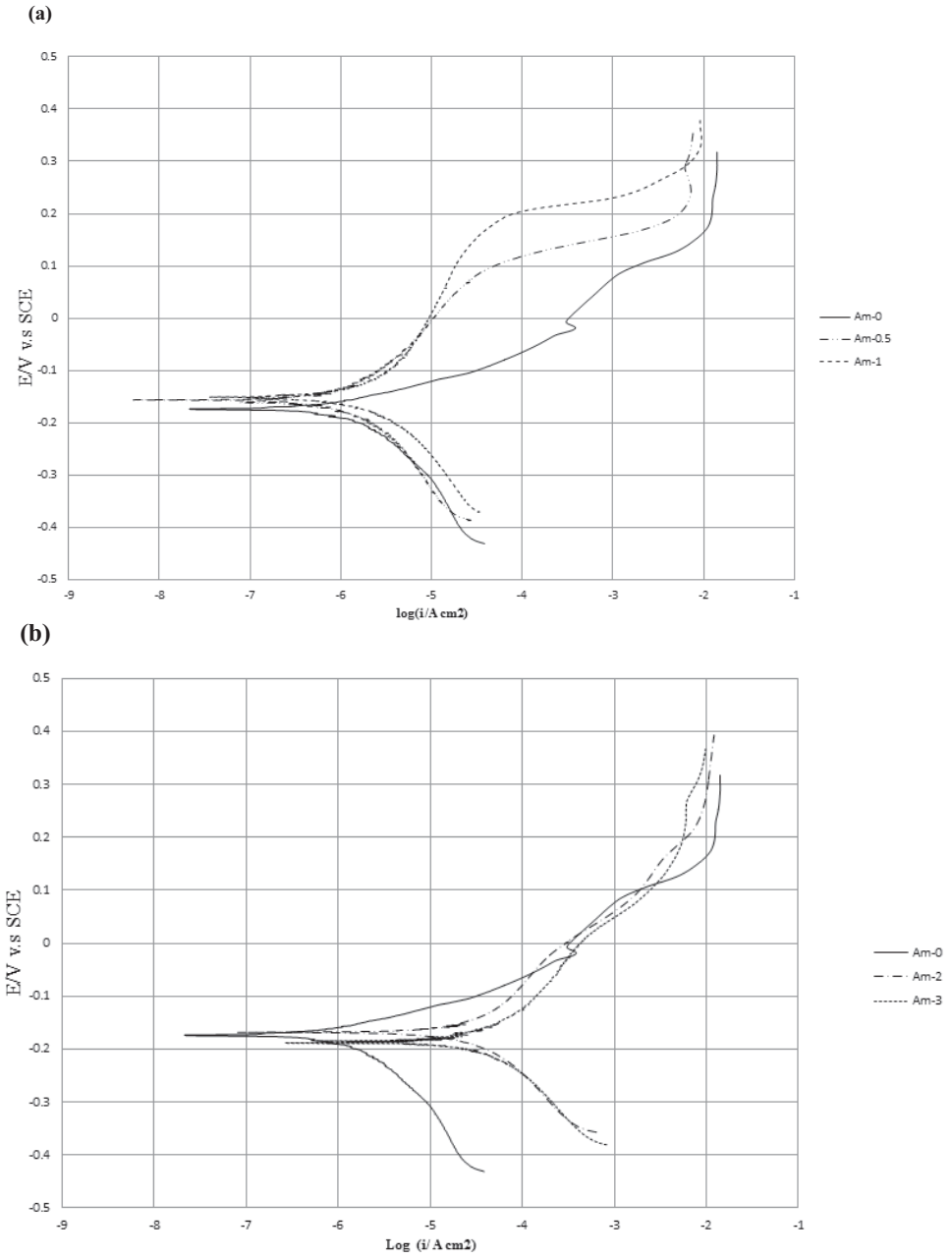

Fig. 6. Representative anodic potentiodynamic polarization curves of reference dental amalgam with (a) dental amalgams containing 0.5 and 1 wt% TiO_2 NPs and (b) dental amalgams containing 2 and 3 wt% TiO_2 NPs.

amalgams containing admixed indium or noble metals have higher compressive strength [10, 14]. In this research, it confirmed that TiO_2 NPs (up to 1 wt. %) improve compressive strength of dental amalgam. It is related to amalgamation process affecting by the present of high amount of TiO_2 NPs. By adding of TiO_2 NPs to amalgam until 1 wt. %, amalgamation process is done easily but by increasing TiO_2 NPs, the nanoparticles is acted as barrier and not allowed to trituration is performed well. So the amalgamation process faces disturbance and

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Table 2. Mean values (standard deviation) of corrosion current densities and corrosion potentials.

Sample code	**Additive (wt.%)**	i_{Corr} **(μA/Cm^2)**	E_{Corr} **(mV)**
Am-0	0	0.71 (0.050)	-198
Am-0.5	0.5	0.77 (0.066)	-146
Am-1	1	0.92(0.077)	-132
Am-2	2	11.2 (0.640)	-170
Am-3	3	16.3 (0.693)	-185

Table 3. Mean values (standard deviation) of compressive strength.

Sample	**Mean values (standard deviation) of compressive strength (MPa)**
Am-0	310(10)
Am-0.5	328(12)
Am-1	365(14)
Am-2	232(13)
Am-3	208(12)

hence the compressive strength decreases.

The results of the antibacterial tests against Streptococcus mutans in the presence of amalgams are illustrated in Figure 7. Means as a whole were considerably different at the 5% level. As seen in the curve, for control sample the optical density was progressively increased during 24 hours, while for reference dental amalgam and dental amalgam containing TiO_2 NPs, there is an initial reducing and next an increasing in optical density. It means that growth of bacteria decrease during the first four hours and it can be related to compatibility of bacteria in new environment. So, optical density and consequently the growth reduce. For the period time of 4 to 24 hours, optical density increase but at all times it is lower than control sample. According to the previous researches, this inhibition of growth is likely caused by the release of metallic ions from the surfaces of the dental amalgams [19, 21, 22]. It is generally admitted that the constituents of these materials (Ag, Cu, Sn, and Hg) have antibacterial properties so that Hg and Cu have more antibacterial properties than Ag and Sn against Streptococcus mutans [22]. Hence releasing of metallic ions is the main reason for decreasing of optical density in samples but the present nanoparticles of TiO_2 in dental amalgam led to declining growth more than reference dental amalgam. It can be attributed role of TiO_2 NPs in antibacterial activity of dental amalgam and it enhances the antibacterial properties of dental

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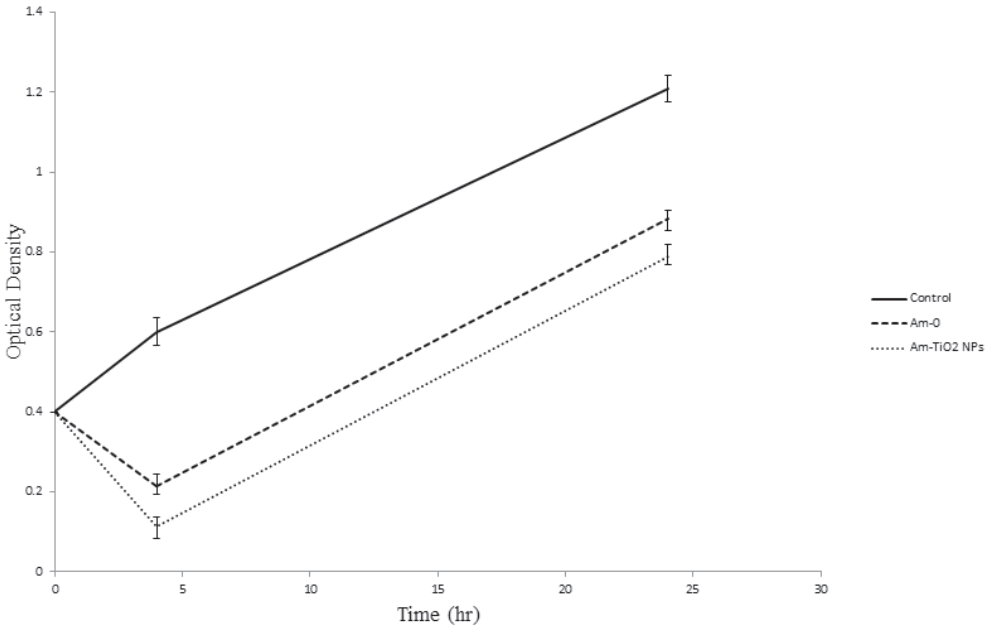

Fig. 7. Optical density vs. time curve for Streptococcus mutans growth.

amalgam.

4. CONCLUSIONS

This is a new investigation about the synthesis of amalgam/ TiO_2 NPs nanocomposite. The results showed that adding 1 wt. % TiO_2 NPs can improve the compressive strength of the dental amalgam. In addition, the results indicated that corrosion current density in nanocomposite consisting of 1 wt. % TiO_2 NPs and lower than it, changes a little while by increasing more than one present additive, corrosion current density changes significantly. Also, the results of antibacterial tests proved that the dental amalgam containing TiO_2 NPs have more antibacterial activity and inhibit the growth of bacteria more than the reference dental amalgam. Hence, it can be concluded that the small amounts of TiO_2 NPs is suitable and seems to be a promising action to improve the properties of this dental material. The results of present study suggest that amalgam/ TiO_2 NPs nanocomposite with 1% of TiO_2 NPs could be regarded as a biocompatible and bioactive dental material that provide better characters for dental applications.

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