

## THE EFFECT OF ADDING TiO<sub>2</sub> 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<sub>2</sub> nanoparticles and evaluation of their compressive strength, antibacterial and corrosion behavior. In this experimental research, TiO<sub>2</sub> nanoparticles (TiO<sub>2</sub> NPs) were added to reference amalgam alloy powder and then, dental amalgam was prepared. In order to investigate the effect of TiO<sub>2</sub> NPs on properties of dental amalgam, 0, 0.5, 1, 2 and 3 wt. % of TiO<sub>2</sub> 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<sub>2</sub> NPs is similar to the corrosion behavior of the reference amalgam, while with increasing the weight percent of TiO<sub>2</sub> NPs, the corrosion rate increases. Also, the results of this investigation indicated that adding TiO<sub>2</sub> 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<sub>2</sub> NPs can increase the biocompatibility and antibacterial activity of dental amalgam. The results of present study suggest that amalgam/ TiO<sub>2</sub> NPs nanocomposite with 1% of TiO<sub>2</sub> NPs could be regarded as a biocompatible and bioactive dental material that provide better characters for dental applications.

**Keywords:** Dental amalgam, TiO<sub>2</sub> 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

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<sub>2</sub> 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 decreasing size of particles so TiO<sub>2</sub> nanoparticles (TiO<sub>2</sub> NPs ) have more antibacterials properties than micropowder of titania [16-18].

The aim of present research was fabrication and characterization of amalgam/TiO<sub>2</sub> NPs nanocomposite and evaluation of its corrosion behavior, antibacterial activity and compressive strength. Therefore, in this study TiO<sub>2</sub> NPs with varying amounts were added to amalgam alloy and mentioned properties of the amalgam/ TiO<sub>2</sub> NPs nanocomposites was compared to the reference dental amalgam.

## 2. EXPERIMENTAL PROCEDURE

Characterizations of used dental amalgam and TiO<sub>2</sub> 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 software and was <60 μm. The amounts of 0.5, 1, 2 and 3 weight percent of nanoparticles of TiO<sub>2</sub> NPs were added to amalgam alloy powder and were prepared Am-0, Am-0.5, Am-1, Am-2 and

Am-3 nanocomposites, respectively. 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<sup>-1</sup>. 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 <sub>2</sub> NPs | Evonike   | 21 nm         | TiO <sub>2</sub>                 | Germany      |
| TiO <sub>2</sub>     | Merck     | 1-5 μm        | TiO <sub>2</sub>                 | Germany      |

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  $4.0 \pm 0.1$  mm and  $8.0 \pm 0.1$  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  $37 \pm 1^\circ\text{C}$  for 24 hours. Afterwards, the compressive strength test was done.

In order to evaluation of antibacterial activity of reference dental amalgam and amalgam/  $\text{TiO}_2$  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  $10 \times 6 \times 2$  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  $37^\circ\text{C}$  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 evaluation phases in the reference amalgam and the new amalgam with different amounts additive and scanning was done from  $10^\circ$  to  $80^\circ$  ( $2\theta$ ) in a step size of  $0.02^\circ$ . Scanning Electron Microscopy (SEM) and Energy diffraction Spectroscopy. X-ray Diffraction (EDS; Xpert, Philips, Holland) with Cu target ( $\lambda = 1.542\text{\AA}$ ) was done from  $10^\circ$  to  $80^\circ$ . 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  $\text{TiO}_2$  NPs as starting materials. The phase evaluation showed that amalgam alloy powder has only  $\text{Ag}_3\text{Sn}$  and  $\text{Cu}_3\text{Sn}$  as well as,  $\text{TiO}_2$  NPs contain two phases of  $\text{TiO}_2$  (Anatase and Rutile). The XRD results of reference dental amalgam and dental amalgams with different percent of  $\text{TiO}_2$  NPs are shown in Fig. 2.a. It confirmed the presence of  $\text{Cu}_6\text{Sn}_5$  ( $\epsilon$ ),  $\text{Ag}_3\text{Sn}$  ( $\gamma$ ),  $\text{Ag}_2\text{Hg}_3$  ( $\gamma_1$ ) phases and amount of  $\text{Ag}_4\text{Sn}$ . Indeed, after mixing with mercury, there would be unreacted phase of  $\text{Ag}_3\text{Sn}$ , new phase of  $\text{Ag}_2\text{Hg}_3$  and most of  $\text{Cu}_3\text{Sn}$  convert to  $\text{Cu}_5\text{Sn}_6$

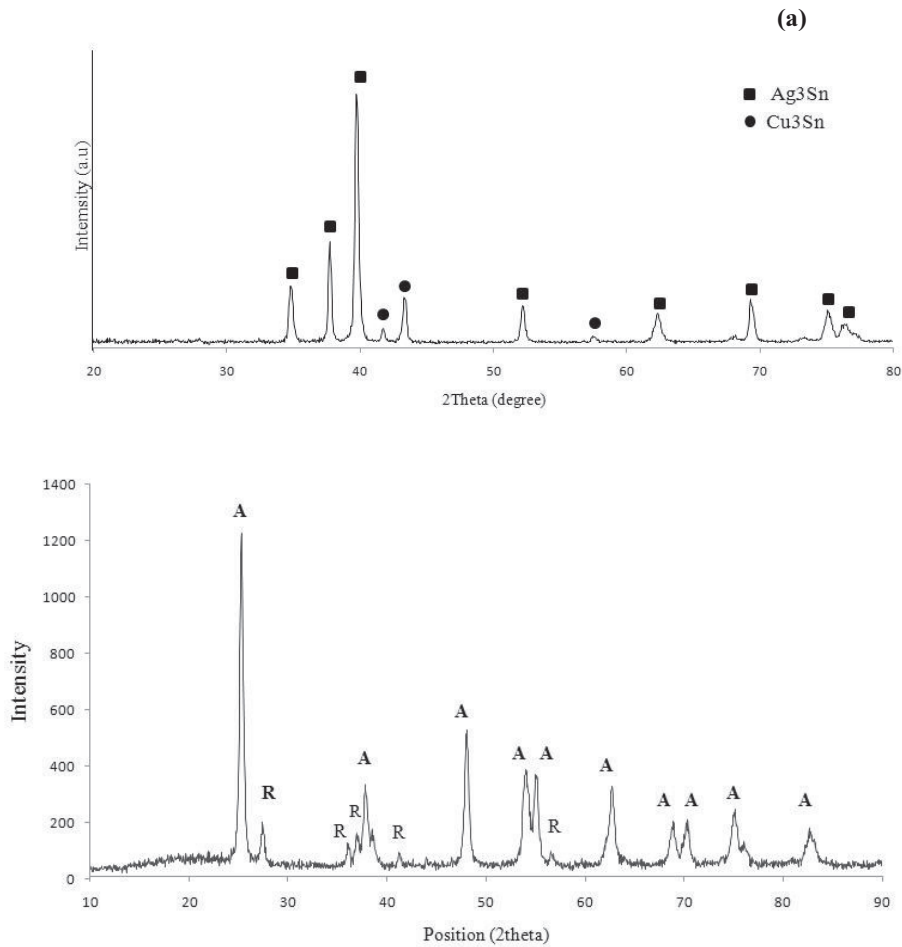
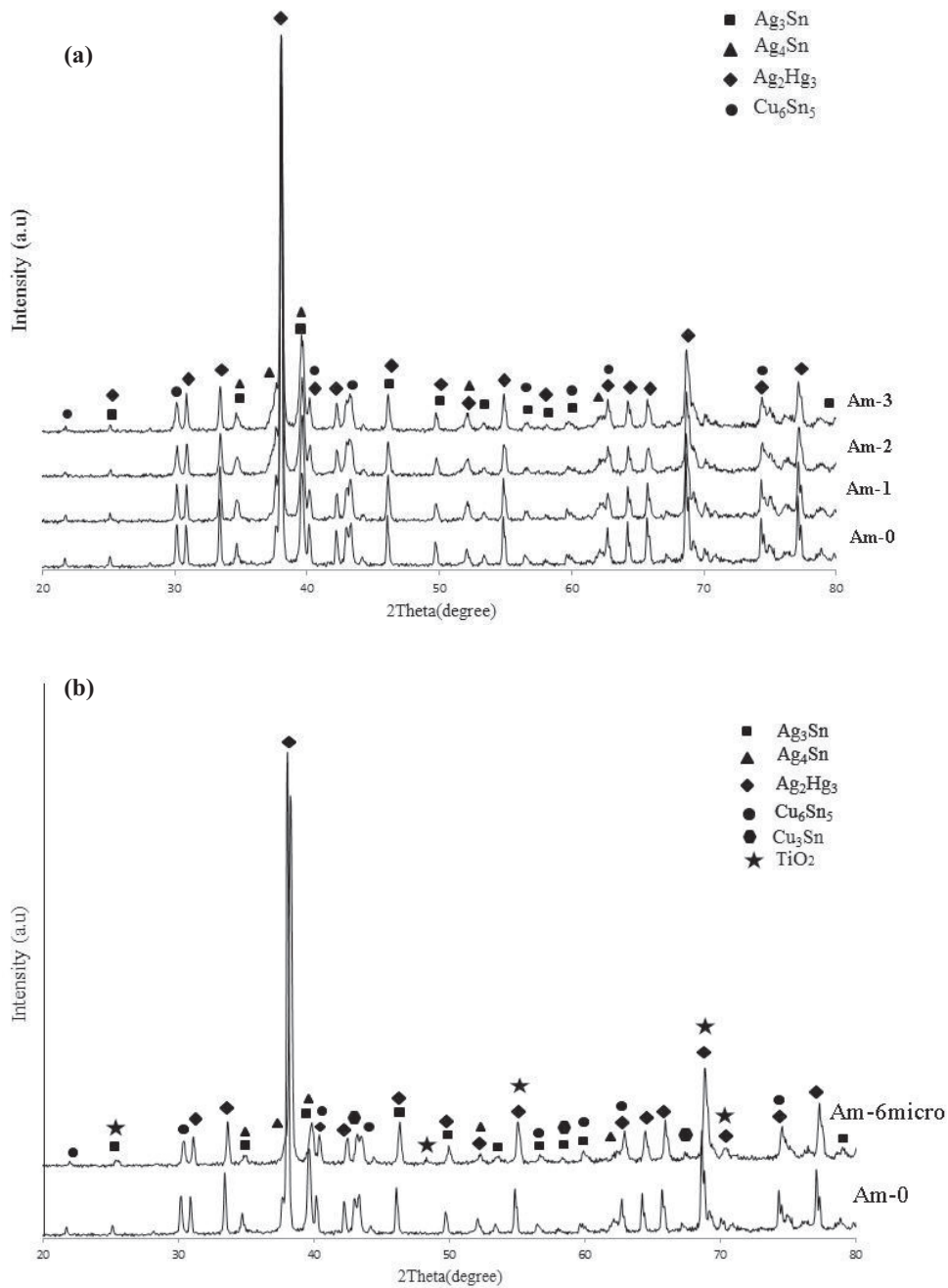


Fig. 1. XRD pattern of a) amalgam alloy powder and b) TiO<sub>2</sub> NPs.

[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<sub>2</sub> NPs no significant differences were identified by adding TiO<sub>2</sub> NPs and they comprise the phases present in the reference amalgam. In addition to, it seems that main peaks of TiO<sub>2</sub> NPs had been placed near to peaks of amalgam. Thus, identifying of TiO<sub>2</sub> NPs is difficult. So, for better evaluation, we prepared a sample with 6wt% micropowder of TiO<sub>2</sub> (Fig 2.b). It should be noted that the maximum amount of TiO<sub>2</sub> NPs as an additive to dental amalgam was 3wt% but it had been 6 wt. % for TiO<sub>2</sub> micropowder. It is due to the very

small particles and high specific area of TiO<sub>2</sub> NPs, so that the amalgamation process faces disturbance in higher level than 3wt. % TiO<sub>2</sub> NPs. This state could make when TiO<sub>2</sub> micropowder add to dental amalgam in higher amounts of 6 wt. %. As can be seen important peaks of TiO<sub>2</sub> had overlapped with dental amalgam phases and there is one peak in  $2\theta=48.2$ , just for TiO<sub>2</sub>. Subsequently, it can be said that TiO<sub>2</sub> 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  $\mu\text{m}$ . The back-scattered electron scanning images of reference amalgam is shown in Figure 3.b and different regions with different phases were

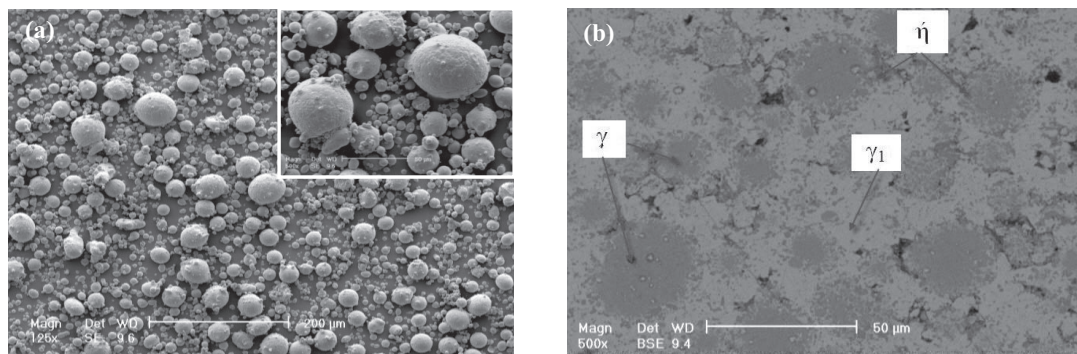


**Fig. 2.** XRD results of reference dental amalgam with (a) dental amalgams containing 1,2and 3 wt% of TiO<sub>2</sub> NPs and (b) dental amalgam with 6wt% micro TiO<sub>2</sub>.

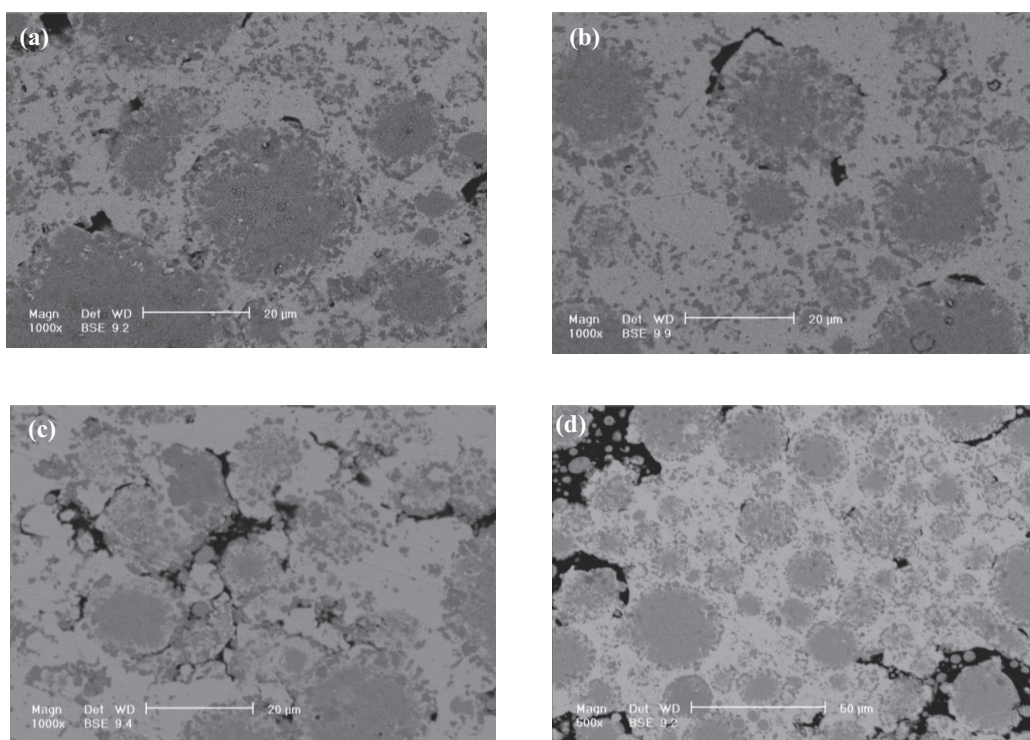
marked. In Figure 4.b dental amalgams with different percent of TiO<sub>2</sub> NPs are indicated. As it can be seen both of the reference amalgam and amalgam/TiO<sub>2</sub> NPs nanocomposites contain Ag<sub>3</sub>Sn ( $\gamma$ ), Ag<sub>2</sub>Hg<sub>3</sub> ( $\gamma$ 1) and Cu<sub>5</sub>Sn<sub>6</sub> ( $\eta$ ). Also, it has been achieved a homogeneous distribution of

phases and did not have any change in phase distribution by adding TiO<sub>2</sub> NPs.

The EDS results of dental amalgam with 1.5 weight percent TiO<sub>2</sub> NPs are indicated in Fig. 5. Also, the EDS spectrum of this area was shown (Fig. 5b). In order to better assess, three regions



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

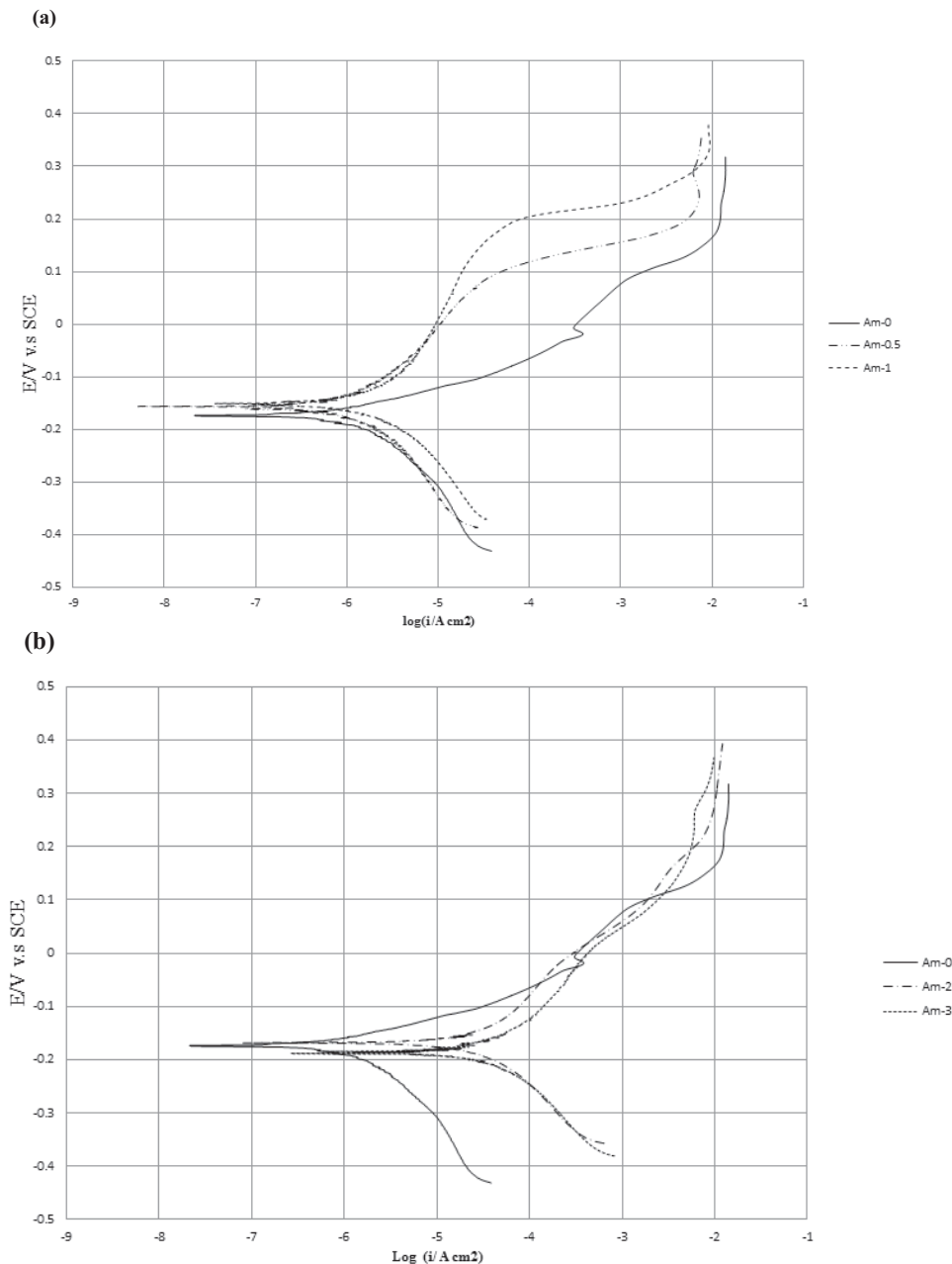


**Fig. 4.** SEM micrographs of amalgam/ TiO<sub>2</sub> NPs nanocomposites (a) 0.5 wt. % , (b) 1 wt. % , (c) 2 wt. % and (d) 3 wt. % of TiO<sub>2</sub> NPs.

were selected: (1) First region contains all of present phases in the dental amalgam; (2) second region contains mainly  $\gamma_1$  and (3) Third region largely contains  $\gamma_1$  and  $\eta$ . 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  $\gamma$  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  $\gamma_1$ , so it would be more mercury in the EDS analysis of this area. Also, there was almost 1.6





**Fig. 6.** Representative anodic potentiodynamic polarization curves of reference dental amalgam with (a) dental amalgams containing 0.5 and 1 wt% TiO<sub>2</sub> NPs and (b) dental amalgams containing 2 and 3 wt% TiO<sub>2</sub> NPs.

amalgams containing admixed indium or noble metals have higher compressive strength [10, 14]. In this research, it confirmed that TiO<sub>2</sub> 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<sub>2</sub> NPs. By adding of TiO<sub>2</sub> NPs to amalgam until 1 wt. %, amalgamation process is done easily but by increasing TiO<sub>2</sub> NPs, the nanoparticles is acted as barrier and not allowed to trituration is performed well. So the amalgamation process faces disturbance and

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

| Sample code   | Additive (wt.%) | $i_{\text{Corr}}$ ( $\mu\text{A}/\text{cm}^2$ ) | $E_{\text{Corr}}$ (mV) |
|---------------|-----------------|---|------------------------|
| <b>Am-0</b>   | 0               | 0.71 (0.050)                                    | -198                   |
| <b>Am-0.5</b> | 0.5             | 0.77 (0.066)                                    | -146                   |
| <b>Am-1</b>   | 1               | 0.92(0.077)                                     | -132                   |
| <b>Am-2</b>   | 2               | 11.2 (0.640)                                    | -170                   |
| <b>Am-3</b>   | 3               | 16.3 (0.693)                                    | -185                   |

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

| Sample        | Mean values (standard deviation)<br>of compressive strength (MPa) |
|---------------|---|
| <b>Am-0</b>   | 310(10)   |
| <b>Am-0.5</b> | 328(12)   |
| <b>Am-1</b>   | 365(14)   |
| <b>Am-2</b>   | 232(13)   |
| <b>Am-3</b>   | 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  $\text{TiO}_2$  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  $\text{TiO}_2$  in dental amalgam led to declining growth more than reference dental amalgam. It can be attributed role of  $\text{TiO}_2$  NPs in antibacterial activity of dental amalgam and it enhances the antibacterial properties of dental

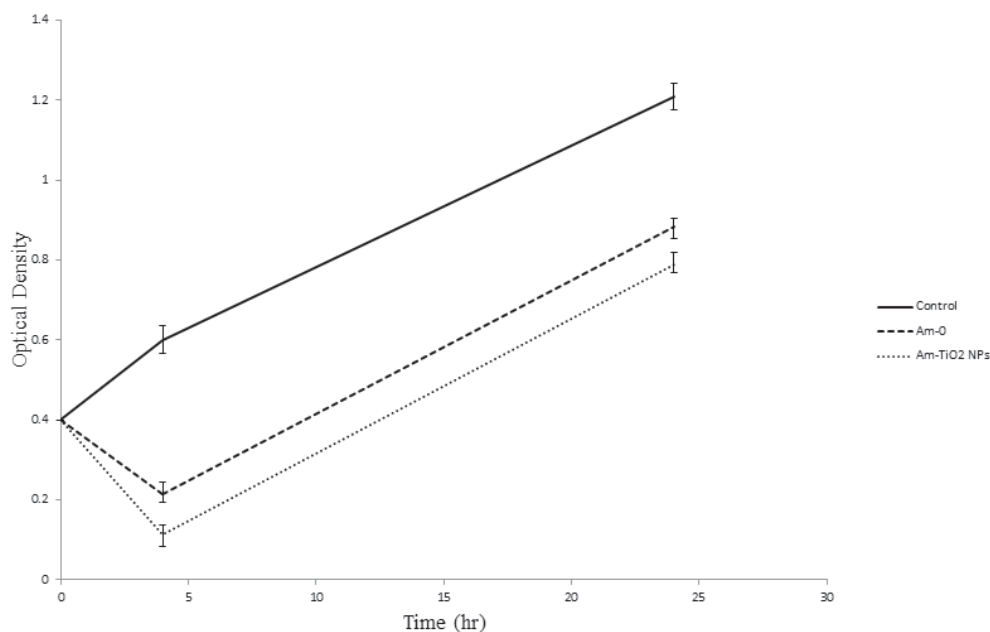


Fig. 7. Optical density vs. time curve for *Streptococcus mutans* growth.

amalgam.

#### 4. CONCLUSIONS

This is a new investigation about the synthesis of amalgam/ TiO<sub>2</sub> NPs nanocomposite. The results showed that adding 1 wt. % TiO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> NPs nanocomposite with 1% of TiO<sub>2</sub> NPs could be regarded as a biocompatible and bioactive dental material that provide better

characters for dental applications.

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