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The Effect of Titanium Dioxide Nanoparticles on Salivary Alkaline Phosphatase Activity

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Abstract

The structural and optical properties of the titanium dioxide nanoparticles [TiO₂ NPs] have been investigated using [UV-Vis] spectrophotometer and SEM. The produced nanoparticles show small and about sharp round peaks around 220 nm. The produced nanoparticles have a spherical shape with an average particle size <50 nm. The effect of titanium dioxide NPs was studied on the activity of Alkaline Phosphatase [ALP] in the saliva of 25 patients with gingivitis in comparison to 20 healthy subjects with the average age about 22–23 years for both groups. The results correlated with the observation that salivary alkaline phosphatase activity increase in patient with gingivitis in comparison to control group and salivary ALP activity inhibited by titanium dioxide nanoparticles.

Keywords: TiO₂ nanoparticles, ALP activity, gingivitis, saliva.

Introduction

Periodontal diseases are bacterial infections of the gingiva, bone and attachment fibers that support the teeth and hold them in the jaw. The main cause of the diseases is a bacterial plaque, a sticky, colorless microbial film that constantly forms on teeth [1]. The microbial plaque accumulation on teeth surfaces adjacent to the gingival tissues brings the oral sulcular and junctional epithelial cells into contact with the waste product, enzymes and surface components of colonizing bacteria. As the bacterial load increases so do the irritation of the host tissues by these substances [2]. From the periodontal diseases gingivitis and periodontitis are the two predominant periodontal diseases and may be present at the same time. In gingivitis, the gingival are inflamed but their destruction is reversible while in periodontitis the inflammatory changes are irreversible changes [3]. Gingivitis represents the inflammation process limited to the gingival tissue next to the tooth without any loss of attachment and possibly acts as a precursor to attachment loss around the tooth [4]. Gingivitis is typically characterized as a vigorous inflammatory response limited

mostly to the superficial gingival connective tissues and is a comparatively nonspecific response to a nonspecific build up of dental plaque. With continuing plaque accrual, gingivitis becomes well established, but still confined to the superficial gingival connective tissues [5].

In humans, alkaline phosphatase [ALP] exists in each tissue through the entire body, but is specially concentrated in liver, bile duct, bone, kidney, the placenta and intestinal mucosa [6]. The enzyme ALP plays a role in bone metabolism. It is a glycoprotein enzyme that is bounded to membrane and it produced by many cells, such as leukocytes, polymorphonuclear, macrophages, fibroblasts, and osteoblasts in the area of the gingival tissue and periodontium [7]. Gao J. et al. [8] show that the highest ALP activity was found in osteoblasts, but moderate in periodontal ligament PDL fibroblasts, and lowest in gingival fibroblasts. No activity was detected in cementoblasts. In the periodontium, the ALP is very important enzyme as it is part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis. Some forms of enzyme are also produced by plaque bacteria [9]. Some studies have shown a strongly increased activity of salivary alkaline phosphatase in periodontal disease particularly in the acute phase, and after the periodontal rehabilitation, the activity of these enzymes restored to the normal value that found in the healthy persons [10].

Nanoparticles have many different effects on human health relative to bulk original material from which they are produced [11]. Consequently of their tiny size, nanoparticles may propose other advantages to the biomedical field through increased biocompatibility [12]. Studies of the effects of nanoparticles [NPs] from different industry branches on cells and pathways are emerging, and most of the biological effects of NPs seem to be due to their interactions with proteins [13]. Enzymes are biologic polymers that catalyze the chemical reactions that make life possible, with the exception of catalytic RNA molecules, or ribozymes, enzymes are proteins [14].

In current years, metal oxide nanoparticles have been mainly examined for their antimicrobial activity, especially titanium dioxide which is considered a clean photo catalyst, low-cost, with nontoxicity and chemical stability [15, 16]. Titanium dioxide nanoparticles are produced universally for multiplicity of engineering and bioengineering uses. Titanium dioxide nanoparticles are often consumed as a material for orthopedic implants. TiO₂ in powder form is also commonly used as a whitener in toothpastes [15, 16]. Titanium dioxide is the most extensively used white pigment because of its brilliance and refractive index for it which is very high, in which it is exceeded just by a few other materials. About 4.6 million tons of pigmentary TiO₂ are used yearly, and this number is likely to increase as application continues to rise [17].

There is no study about the effect of TiO₂ NPs on ALP activity in saliva of gingivitis patients, so we decided to investigate this effect in this research.

Material and methods

1. Nanoparticles

Titanium dioxide nanoparticles have been obtained from Hongwunanmter, China. This product supplies as TiO₂ Nano powder. Absorbance spectra of NPs solution were measured by UV-VIS spectrophotometer. All spectra were measured at room-temperature in a quartz cell with 1 cm optical path. The structure and nano size measurement of nanoparticles samples were identified by the Scanning Electron Microscope SEM (Electronic Microscope Centre-Collage of applied Science, University of Technology, Iraq).

2. Salivary Alkaline Phosphatase assay

Alkaline phosphatase activity was measured to determine the best saliva volume for this experiment by using different volume of sample 20, 40, 60, 80 and 100 μ l. The salivary Alkaline Phosphatase activity was determined spectrophotometrically according to the recommendation of the German Clinical Chemistry Association using the kit of Human Company, Germany. The reaction mixture contained a substrate 50 mmol/L p-Nitrophenylphosphate, and a buffer contained 1.25 mol/L Diethanolamine buffer [pH 10.35 \pm 0.2] and 0.625 mmol/L magnesium chloride in a total volume of 1.250 ml. In the presence of alkaline phosphatase [100 μ L of saliva], p-nitrophenylephosphate is reduced to p-nitrophenol. The rate of decrease in absorbance at 405 nm is directly proportionate to the ALP activity in the sample.

3. Collection of Saliva

Unstimulated saliva of 25 patients with gingivitis from teaching hospital of College of Dentistry/University of Baghdad was collected. The diagnosis of the patients was done by the

following assessment parameters: Gingival index [Loe and Sillness] [18], and Plaque index [Sillness and Loe] [19]. Another 20 healthy samples were collected from dental students with average age about 22-23 years for both groups. Collection was performed during 2-3 hours after the volunteer usual breakfast time and after thoroughly rinsing the mouth with water. Saliva was collected by standard spitting method from both groups: control and gingivitis group then saliva collected in a plane tube, centrifuged 10 min at 1500 g, and the supernatant liquid was used for chemical analysis.

4. Effect of TiO₂ nanoparticles on salivary ALP activity

Stock solution of [200 µg/ml] concentration of TiO₂ NPs was prepared and then the following concentrations 10, 20, 30, 50, and 100 µg/ml are prepared by diluting with the same solvent. The enzyme ALP activity was measured in human saliva by using 100 µl of saliva in the same method with replace 20 µl of the solvent [3:1,water:ethanol] with 20 µl of TiO₂ NPs solution. The percentage effect on activity was calculated by comparing the activity with and without the TiO₂ NPs and under the same conditions according to the following equations:

% inhibition = $100 - 100 \times [\text{Activity in the presence of nanoparticles} / \text{Activity without the nanoparticles}]$

A constant final concentration of TiO₂ NPs [0.15 µg/ml] was used to measure the enzyme activity in saliva samples of gingivitis patients and control persons.

5. Statistical analysis

Statistical analysis was performed using SPSS [version 14] and Microsoft Office Excel [Microsoft Office Excel for windows; 2010]. Data were analyzed by using Two Way Analysis of Variance [ANOVA]. The Student t-test was used to assess significant difference among means at level [P < 0.05].

Results and discussion

Table 1 shows the obvious difference between control group and persons with gingivitis in gingival and plaque index.

Table 1. Summary of GI and PI Scores

	N	Gingival Index [Mean ± SD]	Plaque Index [Mean ± SD]
Control persons	20	0.95±0.20	0.61±0.27
Patient with Gingivitis	25	1.37 ± 0.31	1.59 ± 0.36

Figure 1 shows the difference in mean and standard deviation for plaque index and gingival index in healthy control person [PLI, GI] and patient with gingivitis [PLI₂, GI₂]. The increase in the two main parameters considers from the main causes of gingivitis and this is proved in many studies.

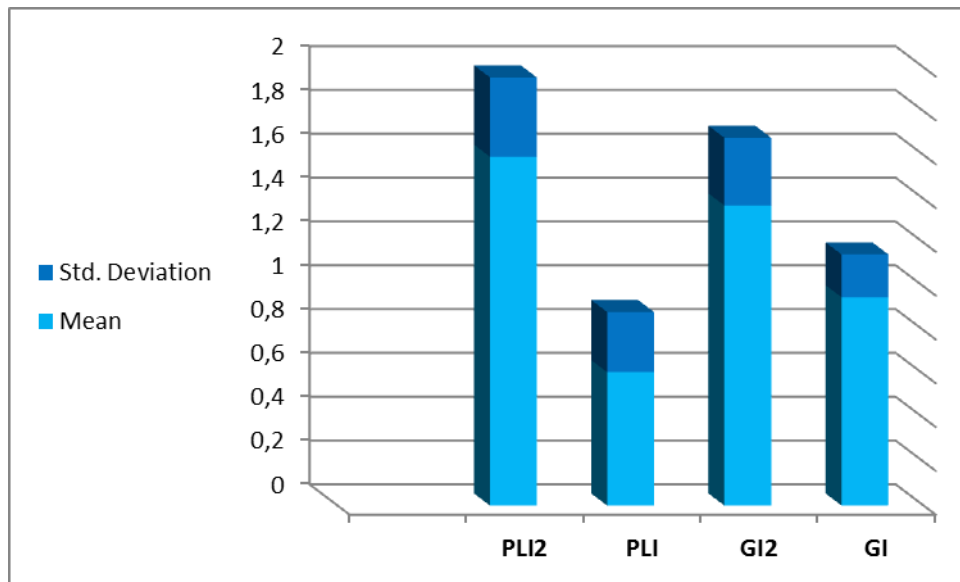


Figure 1. The difference between control in plaque index [PLI] and gingival index [GI] and gingivitis group [PLI2, GI2]

Figure 2 shows the UV-VIS absorption spectra that indicated the characteristic absorbance feature of titanium dioxide nanoparticles, illustrates absorption spectra of titanium dioxide nanoparticles, so the surface related peak could be clearly distinguished. This peak was around 240 nm. This result is in agreement with that of Chen et al [20], which indicate the absorbance intensity of GalA-TiO₂ NPs dispersion in the UV region at <300 nm.

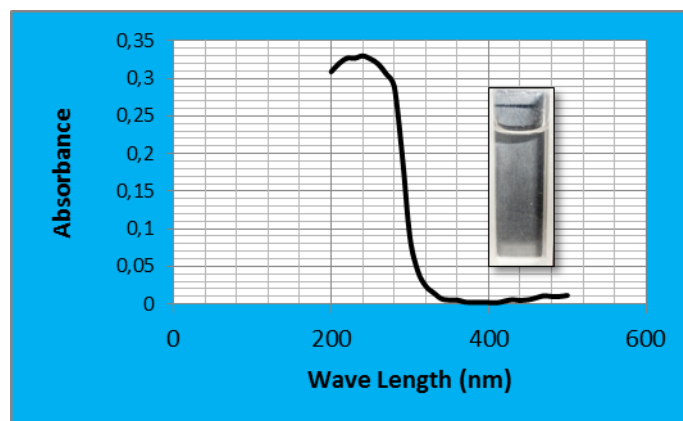


Figure 2. Absorbance spectra of the titanium dioxide nanoparticles

Figure 3 shows SEM pictures and size distributions of titanium dioxide nanoparticles using in this research. The nanoparticles thus produced were calculated to have the average diameters of 30 nm.

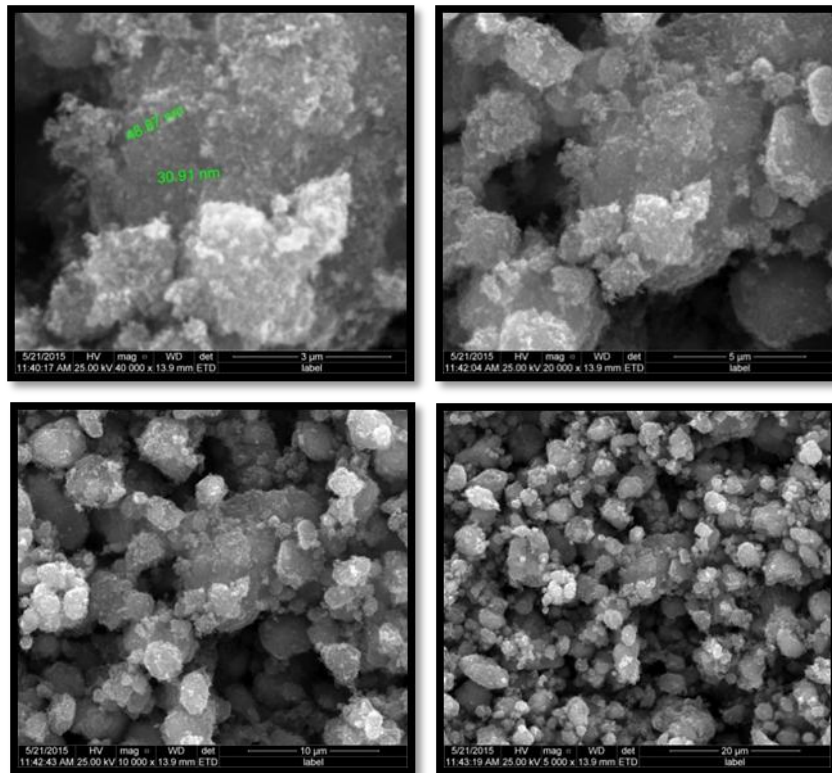


Figure 3. SEM pictures and size distributions of titanium dioxide nanoparticles

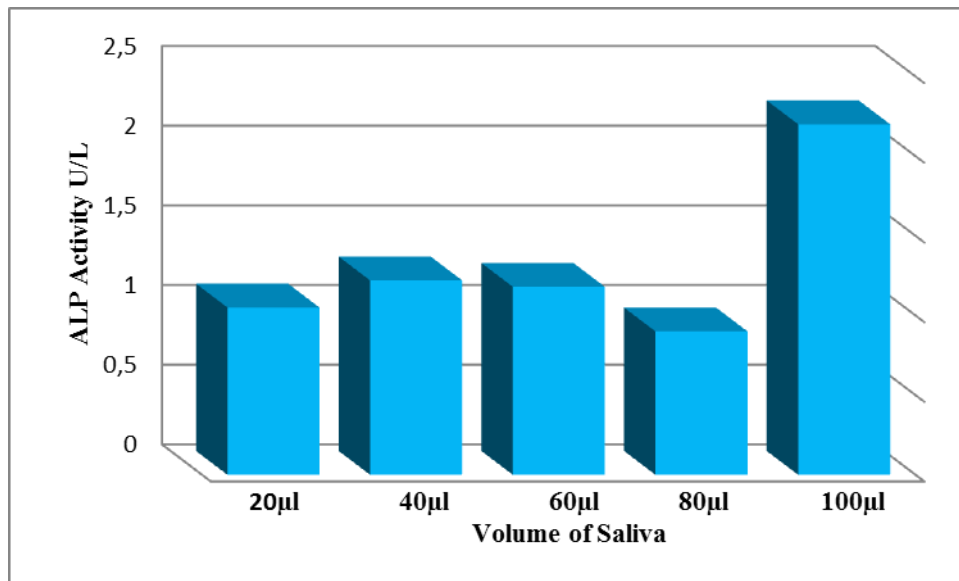


Figure 4. Effect of saliva volume on ALP activity

From the results in Figure 4, it is concluded that 100 µl of saliva is the best volume for measuring the ALP activity according to the conditions of experiments. The kinetic biochemical tests revealed that NPs of TiO₂ caused the inhibition effect on the salivary ALP activity as shown in Figure 5. The observed results show that any increase in TiO₂ NPs concentration caused decreasing in percentage of inhibition of enzyme activity. The greater inhibition of TiO₂ NPs on enzyme activity was at concentration 0.15 µg/ml in total volume 1.370 ml of solvent as shown in Figure 6.

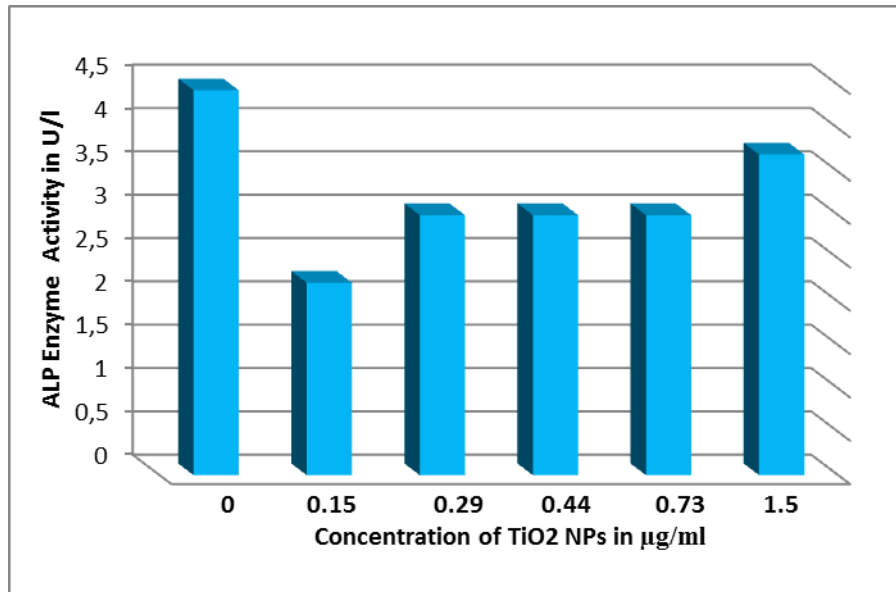


Figure 5. Salivary ALP activity in presence of different concentration of TiO₂ NPs

The greater percentage of inhibition of TiO₂ NPs on enzyme activity was 50 % at concentration 0.15 µg/ml as a final concentration as seen in Figure 6.

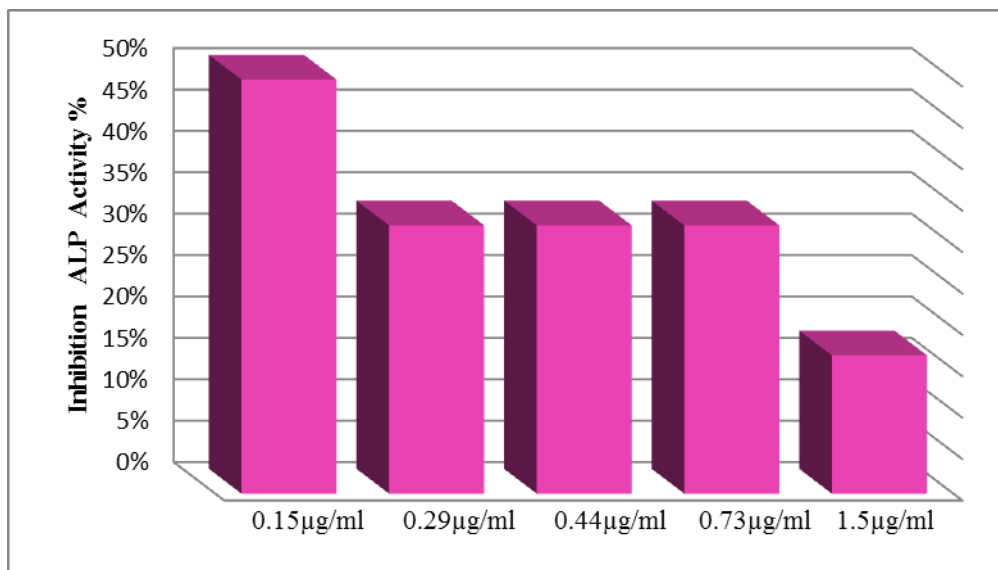


Figure 6. Percentage inhibition of salivary ALP activity in different concentrations of TiO₂ NPs

In recent study of Al-Rubaei et al. [21] on the effect of Au and Ag nanoparticles on acid phosphatase activity, similar inhibition effect was obtained, the greater inhibition of Au NPs on ACP activity in sera of healthy subjects was 5 % at concentration 5.7 µg/ml and Ag nanoparticles was 5.8 % at concentration 10 µg/ml. Another study on the effect of silver nanoparticles on salivary LDH activity observed that any increase in Ag nanoparticles concentration caused increasing in percentage of inhibition of enzyme activity and the greater inhibition of Ag NPs on enzyme activity was 40.1 % at concentration 7.5 µg/ml [22].

Schug et al. results [23] show that in intact heterotrophic biofilms, the alkaline phosphatase activity was not affected following exposure to surface functionalized TiO₂ NPs and UV radiation. While, an alkaline phosphatase enzyme isolated from *E. coli* was powerfully inhibited at lower concentrations of titanium dioxide nanoparticles than the intact biofilms.

We need many studies in order to investigate and explain the cause of high inhibition of TiO₂ NPs on ALP activity in lower range of concentrations, and determine the type of inhibition.

To try to realize the possible effect of TiO₂ NPs on salivary ALP activity in patients with gingivitis the activity measured with and without NPs and compared with that of control group. The results shown in Table 2.

Table 2: Mean and standard deviation for salivary ALP activity in Gingivitis patients with and without TiO₂ NPs and control group

Groups	Salivary ALP Activity in U/L Mean ± SD
Control group	0.74±0.77
Gingivitis group without NPs	1.49±1.34
Gingivitis group with NPs	0.72±0.76

Table 3: Significance level among the three groups*

	df	P value
Contro l - Gingivitis	19	0.036
Gingivi tis - TiO ₂ NPs	19	0.000
Contro l - TiO ₂ NPs	10	0.916

* Control group which represent measurement the salivary ALP activity in healthy subject without TiO₂ NPs and gingivitis group, that represent ALP activity in patients with gingivitis without [TiO₂ NPs] and the third group, salivary ALP activity in patients with gingivitis with [0.15 µg/ml] of TiO₂ NPs as a final concentration

Statistical analysis comparing between the ALP activity of the three groups of this study revealed a significant difference [P< 0.05] between control and gingivitis as the ALP activity increase in gingivitis. This result agree with many results from other studies as in Yoshie et al. [24] that showed an increase in the alkaline phosphatase activity in the severe stage of periodontal diseases, and after treatment, the activity of this enzyme was restored to the normal value found in healthy persons. There are some suggestions to explain this increase. Yan et al. [25] show the increase in ALP enzyme activity may be due to their release by inflammatory cells and bacterial cells into gingival crevicular fluid and consequently into saliva. This result agree with Numabe et al. [26] who show that changes in enzymatic activity of salivary ALP activity reflect metabolic changes in the gingiva in inflammation [26]. While other observations [26, 27] suggest that a significant amount of alkaline phosphatase levels present in saliva is produced locally by diseased periodontal tissues. The ALP levels can be considered as potential indicator for periodontal disease [27, 28]. Mojgan and Afsanehin [29] show that there is unusual conditions, like loss of alveolar bone due to periodontitis, the activity of salivary ALP may demonstrate a significant rise, which is very important biomarker for periodontal problems.

The same statistical analysis comparing between the [gingivitis - TiO₂ NPs] group, a highly significant difference P<0.001 as a result for inhibition of ALP activity by titanium dioxide nanoparticles. While for the third group [Control - TiO₂ NPs] statistical analysis revealed a

nonsignificant difference $P > 0.05$ as the ALP activity increase in gingivitis and inhibited by TiO₂ nanoparticles.

Conclusion

In conclusion, the salivary ALP activity was inhibited, a highly significant, by lower concentration of TiO₂ NPs [0.15 µg/ml] more than the upper range of concentrations and this need another study to explain it. Enzyme levels in saliva of gingivitis patients were significantly high than the control.

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