

The Influence of Titanium Dioxide Nanoparticles Incorporation into Soft Denture Lining Material on *Candida albicans* Adherence and Some Mechanical and Physical Properties

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This study aimed to assess the effect of titanium nanoparticles (TiNP_s) addition into soft denture lining material on *Candida albicans* (*C. albicans*) adherence ability, hardness, shear bond strength and spectrophotometer color absorption of the lining material. TiNP_s with 2% concentration by weight (according to the pilot study) were added into acrylic-based heat cured soft denture liner. One hundred and twenty different specimens were prepared and divided into four groups according to the test to be performed. The ability of *C. albicans* to adhere on the soft liner/ TiNP_s composite was evaluated in three different periods, also hardness, shear bond strength and light absorption by liner material were measured, the results showed there were significant decrease in adhered *Candida* cells number on the surface of the experimental specimens in comparison with the control specimens in each incubation period, and significant decrease in the hardness of the experimental specimens. The strength of the shear bond between the soft liner and the acrylic denture base showed non-significant difference but a significant increase in light absorption percentage was observed in all experimental specimens. Thus it can be concluded that the addition of TiNP_s can provide soft liner material with antifungal properties, reduced hardness and unaffected shear bond strength with increased opacity of the liner material.

Keywords: soft denture liner, *Candida albicans*, titanium nanoparticles.

When the underling residual ridge resorb, the denture will lose its accurate adaptation on this resorbed ridge. In order to improve denture's fitting to the underling supporting tissues, retention, stability, occlusion and patient's appearance, denture should be relined¹.

Soft denture liners act as a layer of absorbing cushion, they can absorb and distribute functional forces which transmitted to the underling irritated, damaged and thin bearing mucosa thus, maintain and restore the integrity of these inflamed tissues. Mostly soft denture liners are used for patients who cannot tolerate the hard base of their complete or partial dentures, so the liner material

will make denture wearing more comfortable and accepted by them².

Also by application of soft denture liners, this can enhance patient's masticatory ability, biting forces and chewing efficiency by improving denture retention³.

However, these liners have many of drawbacks which appear during their clinical use and affect adversely on their serviceability by altering their structure and properties⁴, one of the most important drawbacks of these liners, the colonization of such materials by pathological microorganisms especially *C. albicans* which can adhere and go to penetrate inside the material causing denture induced stomatitis or denture sore mouth⁵.

Denture stomatitis etiology is multifactorial and despite these etiological factors,

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C. albicans has been considered the primary pathological microorganism that most widely isolated from the oral cavity of patients suffering from denture stomatitis⁶.

Furthermore, they have been found that these materials were more prone to colonization by microorganisms than acrylic denture base materials and this attributed to their rough surface and the physical/chemical affinity with the microorganisms⁷.

Many methods had been used in attempts to clean and disinfect the denture surfaces from the colonized microorganisms which include chemical and mechanical methods, but all these attempts showed an adverse effects on the acrylic denture and the soft lining materials themselves⁸.

Many attempts had been made to overcome all these problems and prevent the colonization of the soft lining material by microorganisms and one of them, was the incorporation of antifungal agents within the soft liner itself and addition of nanoparticles was one of these antifungal agents⁹.

Titanium nanoparticles (TiNP_s) are considered an antifungal agents and they were used in many applications such as treatment of skin infections¹⁰.

In the present study TiNP_s were incorporated into acrylic-based heat cured soft denture lining material in an attempt to minimize the microbial growth of *C. albicans* and evaluating whether this addition would significantly affecting some of the mechanical and physical properties of the soft liner itself.

MATERIALS AND METHODS

A pilot study of *C. adherence* and hardness tests was conducted and three concentrations of the nano material were used (1.5%, 2% and 3%) to select the most appropriate and effective concentration of TiNP_s for each test and according to the results of this pilot study for both tests, 2% of TiNP_s was the most appropriate concentration because 2% concentration showed favorable reduction in *C. albicans* with minimum effect on the mechanical properties of the soft liner material. Therefore, the main study was conducted by addition of titanium nano powder (Sky Spring, USA) with concentration of 2% by weight into polyethyl methacrylate soft denture liner (PEMA)

(Vertex™ Soft, Vertex-Dental, Netherlands). One hundred and twenty different specimens were prepared and divided into four groups according to the tests to be performed.

Scanning electron microscope (SEM S50. FEI, Netherland) was taken for both control (PEMA) and experimental (containing 2% TiNP_s) specimens to show the degree of TiNP_s dispersion within the PEMA matrix.

To investigate the chemical interaction between TiNP_s and PEMA, Fourier Transform Infrared Spectroscopy (FTIR) analysis (IR Prestige-21 Shimadzu, Japan) was conducted.

Fabrication of soft liner specimens loaded with TiNPs to be used in the evaluation of adherence ability of *C. albicans*:

Disk shaped plastic patterns measuring 10mm in diameter and 2mm in thickness¹¹, were used to prepare silicone-stone mold in order to fabricate soft liner specimens. The soft lining material was mixed, packed and cured according to manufacturer's instructions. For experimental specimens TiNPs were added into the liner monomer and dispersed by using probe sonication apparatus (BRANSON, USA) for 3 minutes to break them into individual nanoparticles¹².

The mixture was cooled down by placing the container in a cooling bath (cold-water bath), to prevent bulk heating of the liquid during sonication which can cause substantial liquid evaporation, or the degradation of the material¹³. After complete curing the specimens were finished, polished and autoclaved to be sterile.

Isolation of *C. albicans*

C. albicans can be found in different regions of the human being, but to be more accurate in this study, *C. albicans* was isolated from the oral cavity of 20 patients asking treatment for their complaint which was denture stomatitis with oral thrush¹⁴.

By using a sterile cotton swab, the oral tissue lesions were scrubbed gently and then inoculating a primary isolation medium such as sabouraud dextrose agar (SDA)^{15,16}. These taken swabs were cultured and incubated aerobically at 37°C for 24 - 48 hrs.¹⁷, and can be preserved in 4°C for other investigations and tests.

Identification of *C. albicans*

It was identified by colony morphology as it develops as distinct, creamy, smooth and

convex colonies on SDA^{18,19}, and by microscopical examination using Gram stain method²⁰, furthermore, germ tube formation procedure was used²¹, and the final verification was made by biochemical method by using analytical profile index (API) *Candida* system (bioMérieux).

Evaluating adherence ability of *C. albicans*

To test the antifungal efficacy (AFE) of soft liner/TiNPs composites on the adherence ability of *C. albicans*, *C. albicans* was diluted in 0.9 NaCl and a yeast suspension of about 10⁷ colony forming unit (CFU/ml) (0.5 McFarland standards) was prepared by using a McFarland densitometer²². The sterile soft lining specimens were deposited in sterile bottles containing 20ml of the prepared suspension and incubated for 1hr. at room temperature. After incubation, the specimens removed from the suspension and rinsed twice by phosphate buffered saline solution (PBS) for one minute with gentle rocking to eliminate all the non-adhered cells, dried by filter paper and fixed with methanol, then stained by crystal violet from 30-60 seconds, and rinsed again with (PBS) solution for 30 seconds, then dried with filter paper to be examined under inverted light microscope²³. In four fields of view, adhered cells were enumerated for each specimen. The result expressed as yeast cells/mm². This procedure was repeated after 14 days and 30 days of specimens' storage in artificial saliva at 37°C.

Shore A hardness test

Discs shaped soft liner specimens with diameter of about 30 mm and 3mm thickness²⁴, were prepared in order to measure soft liner's hardness by using Shore A durometer (Time group-TH200, China). The distance between the durometer indenter and the sample was 20mm and the contact time after penetration was 5 seconds, five indentations were made for each sample and their mean were obtained²⁵.

Shear bond strength test

To evaluate shear bond strength of soft lining material to acrylic denture base, acrylic blocks with specified dimensions (75mm × 25mm × 5 mm length, width, depth respectively) with stopper of depth about 3mm needed to be made²⁶. Heat cured acrylic resin (Spofa dental, Czech) blocks were made, mixing, packing and curing was done according to manufacturer's instructions, Each specimen consisting of two blocks of acrylic

base which set over each other forming a space between them measured (25mm × 25mm × 3mm length, width, depth respectively) to be filled with wax. Then the whole specimen was invested into silicon material to fabricate a mold for final specimen curing. Wax elimination procedure was done to be replaced with soft lining material and specimen's curing was carried out.

By using instron testing machine (WDW-20, Laryee Technology Co., Ltd., BEIJING), the specimens were tested and the maximum load required for failure was recorded to calculate the value of bond strength for each specimen according to (ASTM specification D-638m, 1986) formula²⁷.

$$\text{Bond strength (N/mm}^2\text{)} = \frac{\text{Maximum load}}{\text{Cross sectional area}} = \frac{F}{A}$$

Spectrophotometer color absorption

Disk shaped soft liner specimens with 50 ± 1mm in diameter and 0.5 ± 0.05mm in thickness according to ADA specifications No.12²⁸, were used in the measurement of light absorption percentage by using UV-visible spectrophotometer (UV-1800, Biotech engineering management).

Statistical analysis

The results of the study were analyzed by using SPSS (statistical package for social science) computer software (version 21).

The following statistics were employed:

- 1- Descriptive statistic which include:
 - a. Mean
 - b. Standard deviation (S.D)
 - c. Standard error (S.E)
- 2- Inferential statistics:
 - a. Independent t-test.
 - b. One-way ANOVA (analysis of variance) test.

A "P" value of > 0.05 was considered statically non-significant,

d" 0.05 was considered significant and d" 0.01 was considered as highly significant.

RESULTS

FTIR analysis showed that there was no chemical interaction between PEMA and TiNPs (fig.1). SEM images showed that there were fair dispersion and areas of aggregation of some TiNPs in the PEMA (fig.2 A and B). The experimental specimens showed significant decrease in the

number of adhered *Candida* cells on their surface in comparison with the control specimens for each incubation periods of the study (table 1). A non-significant difference between the experimental specimens as the incubation time in artificial saliva increase (fig.3A, B, C and D), (table 2).

There was significant decrease in the hardness of the experimental specimens (table 3), and a non-significant decrease in the shear bond strength after TiNP_s incorporation into the soft liner (table 4), while significant increase in light absorption percentage by the experimental specimens (table 5).

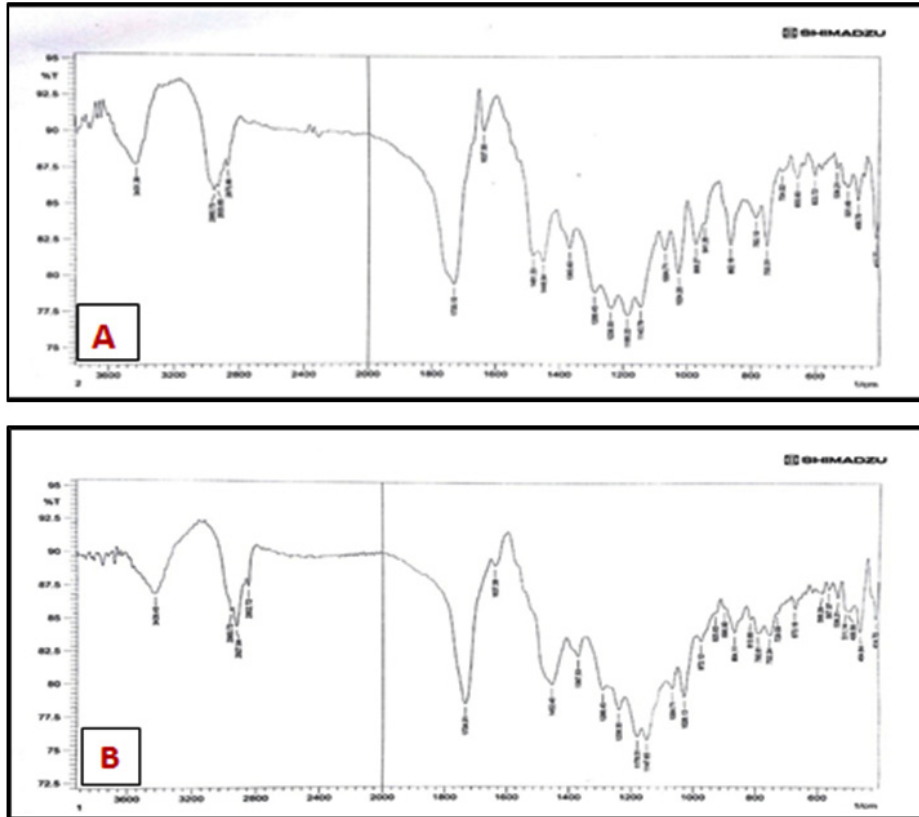


Fig. 1. FTIR spectrum of (A) PEMA, (B) PEMA/TiNP_s

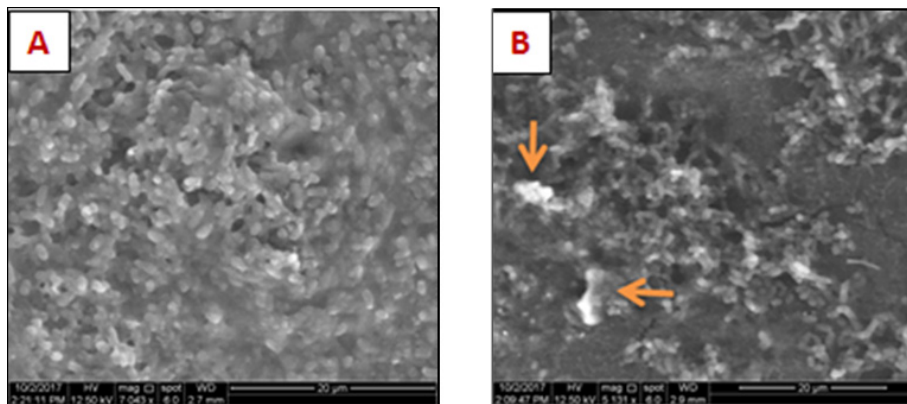


Fig. 2. SEM images for (A) control and (B) experimental specimens

DISCUSSION

In this study an attempt was made to get soft lining material resistant to microbial colonization especially against *C. albicans*. Addition of TiNP_s to the soft liner, should not affect adversely on the mechanical and physical properties of the liner material at the same time

prevent or reduce for a large extent *C. albicans* growth.

The statistical results of this study showed there were highly significant decrease in the numbers of adhered *C. albicans* cells on the surface of the experimental specimens in comparison with the control specimens and this antifungal efficacy of TiNP_s increased with increasing nano

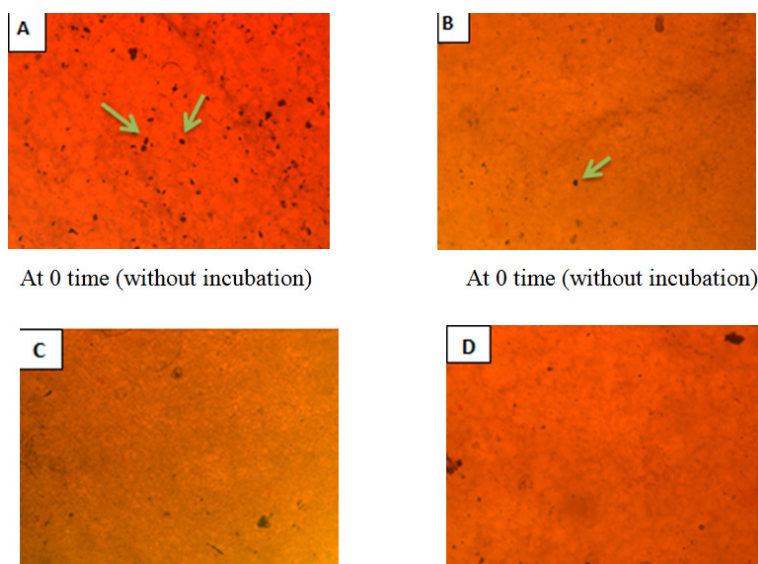


Fig. 3. Fields of experimental and control specimens showing adherent *C. albicans* cells (A) control specimen with a large number of *C. yeast* cells (B), (C) and (D): Experimental specimens with a few number of *C. yeast* cells (10*/0.22)

Table 1. Descriptive statistics and independent t-test showing the difference in the mean values for the studied groups of *C. adherence* test

Groups	Mean	N	SD	SE	t-test	MD	Sig.
Day- (0) control	15.3	10	5.12185	1.61967	6.179	10.30000	0.000
Day - (0) 2%	5	10	1.24722	0.39441			
Day-(14) control	16.7	10	3.65300	1.15518	9 . 1 3 1		
Day-(14) 2%	0.000	4	2.44949	0.77460			
Day-(30) control	15.7	10	5.27152	1.66700	6.729	12.0000	0.000

Table 2. One-way ANOVA for Comparison of the means of *C. albicans* adherence test results for the experimental groups and for different incubation periods of the study.

Groups	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.267	2	4.633	1.202	0.316
Within Groups	104.100	27	3.856		
Total	113.367	29			

concentration. Authors documented that there is an electromagnetic attraction between the microorganism which has a negative charge on its surface and the metal oxide nanoparticle which has a positive charge and by this attraction and surface contact, the microbe will die due to its oxidation²⁹.

It was reported that TiNP_s cause *C. yeast* cells death by production of intracellular reactive oxygen species (ROS) causing oxidation of the Coenzyme A and peroxidation of lipids which decrease respiratory activity and thus cell death³⁰. Another explanation, might be due to the cell membrane tearing of fungi caused by TiNP_s which disturb its integrity, loss of intracellular substances and suppression of the natural budding process through defecting cell cycle at Pre-mitotic phase (G₂/M)³¹.

Soft liner with decreased hardness is preferable because it will has greater softness and thus increased ability to act as absorbing cushion for occlusal forces³².

The results showed a significant decrease in the hardness of the experimental specimens in comparison with the control specimens.

According to the results of SEM of this study, there were areas of agglomeration of some TiNP_s within the acrylic resin matrix because TiNP_s dispersion is difficult due to the presence of strong forces between the particles of the nano material called Van der Waal's forces³³, so the reduction in the hardness may be attributed to this fair dispersion of TiNP_s in PEMA which adversely affects degree of conversion leading to increased level of residual unreacted monomer that acts as a plasticizer^{34,35}, also these agglomerated TiNP_s within the resin matrix can act as stress concentrating centers affecting adversely on the mechanical properties of the polymerized resin material³⁶.

Also the statistical analysis showed that there was a non-significant decrease in the shear bond strength, According to the results of FTIR analyses of this study, no chemical interaction was detected between the TiNP_s and PEMA and this was in agreement with many studies which showed that TiO₂ is inert chemical material and it rarely interacts with other chemical materials so no chemical reaction which may change the chemical

Table 3. Descriptive statistics and independent t-test showing the difference in the mean values for the studied groups of shore A hardness test

Groups	Mean	N	SD	SE	t-test	MD	Sign.
Control shore A hardness	56.9000	10	2.77008	0.87598	2.738	2.86000	0.013
2% shore A hardness	54.0400	10	1.79827	0.56866			

Table 4. Descriptive statistics and independent t-test showing the difference in the mean values for the studied groups of shear bond strength test

Groups	Mean	N	SD	SE	t-test	MD	Sign.
Control shear bond strength	0.4050	10	0.10363	0.03277	0.111	0.00580	0.913
2% shear bond strength	0.3992	10	0.12844	0.04062			

Table 5. Descriptive statistics and independent t-test showing the difference in the mean values for the studied groups of light absorption test

Groups	Mean	N	SD	SE	t-test	MD	Sign.
Control light absorption	2.5940	10	0.33755	0.10674	-11.733	-6.25300	0.000
2% light absorption	8.8470	10	1.65123	0.52216			

SD: Standard deviation, SE: Standard error, MD: Mean difference, df: degree of freedom.

composition of TiO₂ itself or the chemical structure of the other mixed material or the surrounding environment³⁷.

As a result there is no chemical interaction between TiNP_s and the acrylic denture base material (PMMA) and thus the added TiNP_s can act as a barrier that could be interfere with chemical reaction between the PEMA and the PMMA for a little extent especially TiNP_s were mixed with the monomer of the PEMA so may interfere with the monomer ability to diffuse into PMMA. The lining material that used in this study has high ability to flow on the denture base thus enhanced the bond strength between the two bonded materials, also according to the used samples, the surface area between the two materials was large and may help to enhance the bonding strength and this could explain the non-significant decrease in the bond strength³⁸, in addition to the small amount of TiNP_s that added into the lining material.

The statistical results of this study showed a significant increase for UV-light absorption by the experimental specimens, This is attributed to the presence of TiNP_s in the polymer matrix which has good optical and photo catalytic properties and its high ability to absorb UV-light, this type of nanoparticle were able to absorb 95% of UV-light³⁹.

Many studies showed by addition of this nano material will increase the material's life span, maintain its color and esthetic by the ability of TiNP_s to prevent the passage of UV-light, thus reducing the color degradation of the pigments inside the polymer and preventing the color change^{40,41}.

CONCLUSION

The addition of TiNP_s can provide soft liner material with antifungal properties. This addition of nano material lead to reduced liner's hardness with non-significant effect on the shear bond strength but it can increase the opacity of the liner material.

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