

Effect of silver-nanoparticles reinforced resin on Candida growth and fracture resistance of maxillary complete dentures: In vitro evaluation

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Aim: To assess the effect of incorporating silver nanoparticles into acrylic resin denture base material on the Candida growth and fracture resistance of maxillary complete dentures.

Materials and Methods: For this in vitro study, twenty-eight maxillary complete dentures and sixteen samples were constructed from heat cured acrylic resin, where divided into two equal groups. In group I the dentures and samples were conventional (not reinforced) while group II were reinforced with silver nanoparticles with 0.1% of monomer volume, Silver nanoparticles were prepared using Turkevich method and added to monomer during mixing acrylic resin before packing. Fracture resistance test was done using universal testing machine by recording the maximum load before the denture fractures while the samples were inoculated with 20 ml of Candidal suspension and incubated in the incubator for 24 hours in order to evaluate its effect on Candida growth. Difference in colony forming unit count between both groups was evaluated statistically.

Results: The results revealed statistically significant (P-value 0.015), higher fracture resistance of dentures reinforced by silver nanoparticles recording 2320.7 N compared to unreinforced dentures recording 1816.9 N. In addition, revealed statistically significant, less Candida growth on samples reinforced with silver nanoparticles 7.6 +/- 0.24 (log 10) Candida counting forming units compared to 7.9 +/- 0.12 (log 10) in the non-reinforced samples with P-value (<0.001).

Conclusion: The addition of silver nanoparticles 0.1% of monomer volume to acrylic resin denture base material increases the resistance of maxillary complete dentures to fracture and decreases Candida Albicans growth.

Keywords: Silver nanoparticles, reinforcing material, antifungal effect, Maxillary Single denture, denture stomatitis

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Introduction

Although the use of implants has reduced the construction of conventional dentures, still there are many cases that conventional denture is the treatment of choice due to several health and financial reasons.¹ Documented arch discrepancy in tooth survival suggested that maxillary arch exhibits earlier tooth loss compared to mandibular arch. Thus, prosthetic need for maxillary dentures is a common finding. Maxillary complete dentures especially single dentures are accompanied with many complications from which fractures of the denture base are frequently confronted. This may occur because of heavy anterior occlusal contact, or opposing natural teeth exerting excessive occlusal forces or the strong mandibular elevator musculature.²

Polymethyl methacrylate material is frequently used to construct removable dentures because it is biocompatible, acceptable aesthetics, simple processing, relative ease of repair, dimensional stability, stability in the oral environment and low cost.³ Nevertheless, some flaws in the mechanical properties render them non-ideal material for constructing removable prosthesis.¹ Acrylic resin denture bases have certain inherent weaknesses for example its frequent fracture because of low resistance to impact stresses, flexural stresses (Poor denture fit flexes in the mouth during function around the midline), or fatigue stresses (Poor occlusion, repeated small loadings during mastication), low thermal conductivity, and liability to microbial adherence onto the denture inner surface.^{1,4}

Denture fracture is mostly mechanical or accidental that results from flexural fatigue and impact forces. Mechanical reasons are due to improper design, defective fabrication and/or poor materials used. Fracture can be due to a multiplicity of factors rather than the denture base material itself where any condition that exacerbates the deformation of the base or changes its stress distribution will predispose the denture to fracture. For

example areas of stress concentration like large frenal notch, thin or under extended flanges, poorly fitting dentures or lack of adequate relief, dentures having wedged or locked occlusion and repaired dentures.⁵ Continuous force leads to the spread of cracks that reduces the strength of the denture base and finally a midline fracture occurs.¹

To overcome the weaknesses in its mechanical properties and low thermal conductivity, metallic fillers have been incorporated into the conventional PMMA materials with varying success. Reinforcing materials are added so as to advance the mechanical strength of the material of acrylic denture base like carbon fibers, metal strengtheners, nylon fibers, polyethylene fibers with ultra-high molecular weight and glass fibers.⁶ Recently, nanotechnology has encouraged the inclusion of nanoparticles due to their superior properties compared to their macro scale counterparts, simplifying exclusive applications.⁷ This technology allows complete control of the structure of matter at its nano scale dimension.⁸

Nanoparticles improve the mechanical properties such as wear resistance and surface hardness of dental restorative materials. The main difference between nano-metric and micrometric particles is that nanoparticles have significantly larger specific surface area, which greatly facilitates the transfer of load to nanoparticles from the polymer matrix.⁹ Consequently, nanoparticle-reinforced hybrid system exhibits higher stiffness and better resistance to wear. Different types of nano materials like copper, zinc, magnesium, zirconium, titanium, gold and silver have been developed.^{10,11}

The methods of providing AgNPs have different strategies that were successfully used, including physical, chemical, physicochemical, and biological synthesis approaches.¹² Yet, the simplest and safe process, decreased financial implications, and has repeatability and reproducibility of the experimental results,

is the preparation of AgNPs by the chemical reduction of silver salts by sodium citrate or sodium borohydrate.¹³

With the prevalent implementation of nanosilver, still several worries about toxicity and need to be addressed. The incorporation of silver has been strictly limited due to the toxicity of silver ions to humans. Nevertheless, nanotechnology has aided in the production of smaller silver particles with increased large surface area-to-volume ratios, greater efficiency against bacteria and toxicity to humans is markedly lowered.¹⁴ Polar interactions produced among silver nanoparticles and C=O groups of PMMA chains together are the main characteristics which affect the mechanical properties of the material.¹⁵

Silver nanoparticles AgNPs are one of the most commonly used nanoparticles owing to their ductility, thermal and electrical conductivity, and antimicrobial efficiency against bacteria and virus.^{14,15} Silver nanoparticles when added to denture base resin, can increase its flexural strength (ability to resist deformation under load) depending on their concentration so enhance the denture base ability to resist fracture.^{16,17} Besides their antimicrobial effect where silver-nanoparticles works by sensitizing oxygen, catalyzes its conversion to active oxygen that causes structural damage to the membrane of the microorganism.¹⁸

In dentistry, AgNPs are used in the fields of periodontics, restorative dentistry, endodontics, orthodontics, prosthodontics and implantology. They have been principally applied for disinfection and prevention of oral infections owing to their favorable antimicrobial properties.¹⁹ In addition bacteria don't develop resistance against AgNPs, thus they can affect wide spectrum of bacteria. They exhibit an antimicrobial action against Gram-positive and Gram-negative bacteria and fungi.²⁰

Placement of removable prosthesis in the oral cavity produces profound changes of the oral environment that may have an adverse effect on the integrity of

the oral tissues. Mucosal reactions could result from mechanical irritation by the dentures, accumulation of microbial plaque on the dentures, or occasionally a toxic or allergic reaction to the constituents of the denture base material.²

Denture induced stomatitis, also known as denture sore mouth and prosthetic stomatitis, is the most common drawback of prolonged wearing of removable denture. Its etiology is multifactorial, it significantly increase with poor denture hygiene, ill-fitting denture, and microbial colonization atop the inner surface of the denture and oral mucosa.²¹ and systemic factors as diabetes mellitus, malnutrition or defect in host's defense mechanism.²² Its principle pathogens in approximately 90% of cases are *Candida* species, principally *Candida albicans*.¹⁸ Thus the treatments reported are first elimination of predisposing factors, systemic antifungal medication, topical antifungal therapy, maintaining the oral hygiene care, and the denture to be soaked in disinfectants for removal of the source of irritation. Commonly, it is not considered serious, but in the ageing or immune-compromised patients, may cause chronic inflammation and act as a foundation for serious infection.²¹ Thus controlling such infections is of great interest in dentistry, specially in patients wearing complete or partial dentures.²³

Systemic and local application of antifungal medications led to the development of yeast resistance. Yet, the unpleasant taste and inability to keep an effective concentration at the affected site, the inclusion of antifungal and antibacterial agents in material of the denture base is the primary step for denture stomatitis prevention.²⁴

Further studies to verify the antimicrobial efficiency without adverse consequences on physico-mechanical properties upon the inclusion of different nanoparticles into acrylic resin material are still required for efficient clinical performance. Using nanotechnology,

acrylic resins can be promoted to form more effective anti-biofilm and antimicrobial resins in the nano-scales and enhance the long-term use and their physical and mechanical properties. The purpose of using silver nanoparticles is to reduce microbial activity in dental materials by that can endorse oro-dental health and improve the patient's quality of life.²⁵ Thus the aim of this study was to investigate the effect of AgNPs with 0.1% of monomer volume on polymethyl methacrylate acrylic resin to resist its fracture and reduce Candida growth.

Materials & Methods

-Preparation of silver oxide nanoparticles AgNps

Silver oxide nanoparticles were synthesized following the Turkevich¹³ method (Prepared by Egyptian Petroleum Institute (EPRI)) through the reduction of silver nitrate (AgNO_3) with sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) was carried out as previously reported to obtain silver oxide nanoparticles suspension.

The aqueous solution of silver nitrate and sodium citrate was kept at boiling temperature for a couple of minutes until the solution turned amber yellow, indicating the formation of colloidal silver nanoparticles. The produced suspension to be incorporated in the mix within 48 hours to avoid its agglutination and insure proper spread into the liquid monomer.

I- Fracture resistance testing

-Maxillary complete dentures construction

Twenty-eight standard ready made educational models representing completely edentulous maxilla were used for carrying out the complete dentures construction procedures.

Pink modeling wax was adapted on the denture bearing area on each stone model, keeping the space for the placement of rubber base that will simulate the mucosa afterwards. Then bite blocks were constructed and acrylic resin maxillary

teeth were set up following the setting up guidelines. Trial dentures were waxed-up. Thus, twenty-eight sets of maxillary trial dentures having same size teeth were obtained, flaked and wax elimination was carried out.

According to the denture base material, waxed-up dentures were divided into two equal groups;

Group I: Fourteen maxillary heat cured dentures without any reinforcement as a control group

Group II: Fourteen maxillary heat cured dentures reinforced with 0.1% of AgNps of monomer volume of acrylic resin.

The monomer volume in control group (Group I) was 10 ml while in the reinforced group (Group II) was 9.99 ml and 0.01 ml was silver nanoparticles suspension having ratio measured using digital micrometer pipette. Heat cured acrylic resin polymer powder was mixed with the monomer, the acrylic dough of both groups was packed inside the flask, flask was closed and pressed by a Teflon press (Piston). The dentures were deflaked and the excess material was removed and cured according to the manufacturer's instructions. No finishing and polishing were done for the dentures in order not to lose any amount of silver nanoparticles.

-The complete dentures were stored in distilled water for 50 hours at 37°C.

- Construction of acrylic models

Undercuts on the tissue surface of one denture representing each group were blocked out. Improved dental stone was poured into each denture. Each cast was duplicated using self-cure acrylic resin. The surface of the produced model was reduced by grinding 2 mm uniform thickness of its surface. Then a stone key was formed on the surface of the reduced model to keep the space formed for the layer of silicon rubber base uniformly covering the denture bearing area.

The denture bearing area on the acrylic model was painted with rubber base adhesive material and light body rubber

base impression material was injected on the reduced model to simulate the resiliency of oral mucosa where it exhibits visco-elastic behavior during loading. The stone key was painted with separating medium and relocated over the rubber base till setting of the material.

- Assessment of fracture resistance of maxillary complete dentures

Each denture was placed on the prepared cast for the application of load thus will be supported simulating the oral cavity. The maximum load before each denture fracture was recorded using a universal testing machine (LR5K, Lloyd Instruments Ltd, UK). T-shaped crosshead load applicator was used with its vertical end fixed to the machine and its horizontal ends directed bilaterally between second premolar and first molar thus bilateral vertical load was applied at speed of 0.5 mm/min between the second premolar and first molar following Heartwell and Rahn²⁶ as this is the area where most of the masticatory function is performed and ensuring touching bilaterally at the same time for equalization of the applied force preventing lateral rotation of the dentures during testing. Fig (1)



Fig (1): Maxillary denture on the prepared cast placed in the universal testing machine.

The values of the maximum load causing denture fracture in newton were recorded and tabulated for statistical analysis. Fig (2)



Fig (2): Fractured maxillary dentures.

II-__Testing the effect of silver nanoparticles on Candida growth:

- Samples construction

Sixteen samples were constructed with dimensions 30 mm in length, 10 mm in width and 3mm in thickness as recommended by ADA were constructed. They were classified into two groups:

Group I: Eight heat-cured acrylic resin samples (control group).

Group II: Eight heat-cured acrylic resin samples reinforced by 0.1% of monomer volume measured by digital micrometer pipette.

The samples of both groups were constructed using conventional compression moulding technique; in order to prepare the eight samples with the same acrylic resin mix, in the same conditions and in the same curing cycle. This procedure was done using custom-made copper rectangular plate to prevent discrepancies that might occur during their construction. Fig (3)



Fig (3): Copper rectangular plate with eight moulds.

Copper rectangular plate comprising eight equal rectangular spaces (moulds) were painted with a separating medium, and when heat polymerized acrylic resin reached the dough stage, it was packed into

them. For trial packing, the assembly was closed tightly by the six screws, then reopened and any excess acrylic resin was removed by a sharp knife and the moulds were closed tightly for final packing.

A Teflon press (Piston) was used to pack the acrylic resin into each mould. Acrylic resin was processed according to the manufacturer's instructions. First water was boiled and the moulds were inserted into the water for 15 minutes and boiled for 20 minutes. The mould was allowed to cool down slowly in a water bath after polymerization. No finishing or polishing was done to the samples, only the excess material was removed using a sharp cutter to avoid any discrepancies affecting the results. The heat produced during finishing and polishing procedure may affect the dimensional accuracy of the acrylic resin for standardization of the dentures studied. Then they were stored in deionized water at 37°C for 50 hours before testing in order to get rid of the residual monomer which may affect the quality of the acrylic resin material.

- Preparation of Fungal suspension

Single colonies of *Candida albicans* (Microbiology lab., Ain Shams University) from fresh Sabouraud's agar petri plate cultures were inoculated into an enrichment media brain heart infusion broth (BHI) (Lab M Limited, United Kingdom, LOT 114441/36) using calibrated loop. Fungal suspension was adjusted spectrophotometrically to produce 0.5 MacFarland standard density giving a 3×10^6 colony forming unit (CFU)/ml suspension. (the starting basic *Candida* colony count) Fig (4)



Fig (4): *Candida* colony in fresh Sabouraud's agar plate.

- Testing the prevalence of candidal growth:

Each acrylic sample in both groups was inoculated with 20 ml of fungal suspension and incubated in the incubator (Fisher ISOTEMP incubator model 255D) at 37°C to favor candidal growth. Fig (5) After 24 hours of incubation, the test tubes were applied in a shaker to allow uniformity in distribution of *Candida* colony in broth.

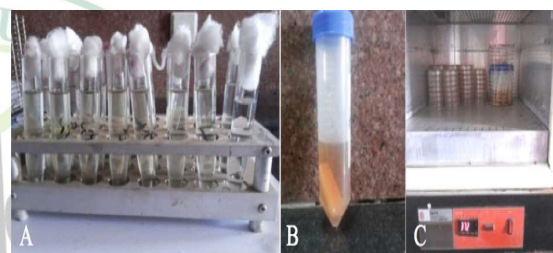


Fig (5): A. Diluted sample suspension, B. Sample inoculated with 20 ml fungal suspension, C. Samples in the incubator

Then the sample suspension was diluted (1/1000) using sterile saline to facilitate colony forming unit count as *Candida* multiply rapidly which makes counting difficult. 1 µl of the diluted sample suspension (1/1000) was collected by calibrated loop and inoculated on Sabouraud agar. (Alpha Chemical, Mumbai, India); a selective media for *Candida* growth and it was supplemented with chloramphenicol for inhibition of bacterial growth.

Colony forming units were counted for samples of both groups and was evaluated statistically. Fig (6)

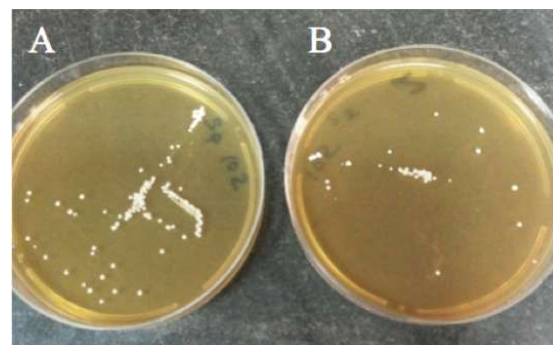


Fig (6): *Candida* colony forming unit results; A. Group I, B. group II

Statistical Analysis

Statistical analysis was performed with IBM (IBM Corporation, NY, USA) SPSS (SPSS, Inc., an IBM Comp) Statistics Version 20 for Windows.

- Numerical data were explored for normality by checking the data distribution, calculating the mean and median values, evaluating histograms and normality curves and using Kolmogorov-Smirnov and Shapiro-Wilk tests.

- Data were presented as means, standard deviation (SD), and standard error values. Independent t test was used to compare forces causing denture fracture between the two tested groups. The significance level was set at $P \leq 0.05$.

Results:

-Fracture Resistance of Maxillary Dentures

Normality test, According to Shapiro wilk test, it was evident that data are normally distributed. This is evident in table (1)

Table (1): Shapiro wilk test for difference, significance and data normality for maximum load (N) at fracture of Group I unreinforced and Group II silver oxide nano-particles reinforced dentures.

Groups	Shapiro-Wilk		
	Statistic	Dentures number*	Significance**
Group I	924	14	503
Group II	846	14	113

*: 14 **: Significant

Maximum load at fracture

The mean values of the maximum load (N) at fracture of Group I the unreinforced resin dentures was 1816.978 N while that of Group II silver oxide nano-particles reinforced dentures was 2320.7 N as presented in table (2). A statistical significance difference P-value 0.015 of maximum load at fracture was evident.

Table (2): Means, Standard deviation and P-value for maximum load (N) at fractures of Group I unreinforced and Group II silver oxide nano-particles reinforced dentures.

Groups	Mean	Standard Deviation	P-values	Significance
Group I	1816.98	473.3	0.015	503
Group II	2320.78	575.7		113

Significance level was set at $P \leq 0.05$.

- Evaluation of Candidal Growth

The mean value of Candida colony count at the beginning of the incubation period was 5.5 (log 10) for both groups' samples while the mean of Candida count for unreinforced samples was 7.9 +/- 0.12 (log 10) and 7.6 +/- 0.24 for the reinforced samples. There were increase in bacterial count in both groups and difference was statically significant P-value (<0.001), this is evident in table (3).

Table (3): Mean values of log 10 of candida CFU count at the beginning of the incubation period and 24 hrs after incubation for unreinforced and nano silver oxide reinforced samples.

Log 10 of Candida colony count	0 hour (Base line)		24 hour After incubation		P value
	Mean	SD	Mean	SD	
Group 1	5.5	0	7.9	0.12	0.001
Group 2	5.5	0	7.6	0.24	<0.001

Significance level was set at $P \leq 0.05$.

Independent t-test Paired t test

Discussion

Among the factors that prominently influence and alter the physical and mechanical properties of dental prosthesis are the denture base material used, the addition of filler particles and the processing method used for polymerization. Nanotechnology has helped to modify the size and shape of the particles in nano scale, provided different means to use these nanoparticles in the diagnosis and treatment of several diseases, and facilitated their application in fields such as bioscience and medicine.

It has been revealed that the performances of several biomaterials used in prosthodontics have been considerably

improved after nanotechnology has reduced their scales, from micron-size into nanosize. The use of silver nanoparticles leads to better processing and a smoother surface, compared to micrometer-sized silver powder thus it is preferred to be added to the acrylic base.^{7, 27}

It is strongly recommended to assess the outcomes of any additive to acrylic materials on their mechanical properties to avoid any adverse effect that may decrease their strength to below standard level.²⁸ The most important causes of denture base fracture are impact failure extraorally and flexural fatigue failure intraorally. The impact strength (IS) and flexural properties of denture base materials are of significance aimed at predicting their clinical performance.¹⁷ Although nanoparticle through attractive interactions can improve the physico mechanical properties of acrylic resin materials, it is recognized that this additive can act as an impurity and affect polymerization reaction.²⁹ Consequently, an appropriate concentration of nanoparticles should be added such that advances the antimicrobial activity of acrylic resins without changing their physico-mechanical properties.^{17, 25}

In the present study samples were used to determine the effect of silver nanoparticles on growth of *Candida albicans* and the maxillary dentures were constructed to assess the ability of silver nanoparticles to enhance resistance against fracture.

In the present study, silver nanoparticles were added with 0.1% of monomer volume where 0.05% show high effect on the flexural strength which enhance the fracture resistance but minimal effect on the adhesion of *Candida albicans* in comparison to polymethyl methacrylate without silver nanoparticles as increasing silver nanoparticles addition to 0.5% and 5% its effect increased in decreasing the candidal adhesion and decreased in increasing on fracture resistance especially

when using AgNPs with 5% not influence its physico- mechanical properties.^{27,30} In another in vitro studies recommended that minimum concentrations of nanoparticles that are effective should be used, since metallic nanoparticles at high weight percentages, affect polymerization of dental materials and cause decrease in their biocompatibility.³¹ And that additional studies are necessary to establish the ideal concentration of AgNPs to ensure optimal antimicrobial effect without cytotoxicity before applying in in-vivo.³²

Three methods have been developed for preparation of nano-composites either mixing nanoparticles with the polymer, generating nanoparticles during polymerization or adding nanoparticles to the monomer.³³ In the present study followed Hamed -Rad et al³⁴ to decrease the agglomeration and get polymer-silver nano-composites, the prepared aqueous solution of AgNPs was dispersed in the acrylic monomer liquid in the predetermined ratio using digital micrometer puppet for accurate measurement then mixed with the powder polymer of acrylic resin.^{17,30,33} This method also makes the distribution of particles be more equal than if placed in a powder form into the polymer powder.

By applying low concentrations of nanoparticles, can reduce material costs and less monomer would be needed while mixing with the polymer, accordingly rendering the procedure cost effective. Consequently, the esthetic appearance and mechanical properties of the cured polymer is at less risk.¹⁶ Because of the plasmon effect of nanoparticles, the acrylic resin united with AgNPs undertook a color alteration, which is believed to be inevitable functional property of base materials.²⁴ The material became darker when the amount of accumulated nanoparticles was higher.²³ AgNPs when added in 0.5% concentration produced a brownish discoloration that isn't a preferred

choice for the aesthetic zones of the denture.³⁵

The addition of silver nanoparticles to complete maxillary dentures increases denture resistance to fracture as shown that group II resists more load before fracture takes place than group I. This finding is in accordance with Monteiro et al.³⁰, Sodagar et al.³⁶ and Castro et al.³⁷ who reported that the addition of silver nanoparticles to acrylic resin increases its flexural strength, that confirms the higher resistance to fracture recorded in group II.

The mechanical properties of acrylic resins were influenced by the concentration in AgNPs where its application in larger amounts as 5% of monomer volume decreases flexural strength and showed very similar mechanical properties with the control group but increases its antimicrobial effect against *Candida albicans*.³⁸ In other studies, the results revealed that the addition of 0.8 and 1.6 wt % and 2 wt % silver nanoparticles changes the cured polymethyl methacrylate.^{28,33} This was explained that nanoparticles ratios act as impurities inside the resin that led to reduction in the mechanical strength of the polymer.^{34,36} Moreover when the dispersion of the nanoparticles in the polymer matrix, decreases the reaction of monomer with the polymer thus the amount of unreacted monomer increases varying according to the reduction of the monomer reaction and it acted as a plasticizer, left over within the matrix that led to decreasing the flexural strength.^{16,30,39} Besides, higher concentrations of silver nanoparticles in the polymer matrix create agglomeration sites that lead to disordered structure of the nanocomposite that negatively affects the mechanical strength of the final product.⁴⁰ Thus was concluded that clinically, adding silver nanoparticles in low concentration enhance acrylic resins mechanical properties.¹⁷

Silver nanoparticles in smaller amount as 0.05% increases flexural strength but decreases antimicrobial effect against

Candida albicans as reported by Monteiro.³⁰ also Sodagar et al.³⁶ found that adding 0.05 wt % of AgNPs is more effective in improving the flexural strength of PMMA denture base material than 0.2 wt % of AgNPs.^{17,28} This study supports the results of the present study as in low concentrations nanoparticles can improve the mechanical properties of denture base materials where the application of 0.1% of silver nanoparticles of monomer volume increases the resistance to fracture besides having an antimicrobial effect against *Candida albicans*, also this was reported by Melo et al.⁴¹

It was found that denture insertion in patient's mouth leads to alteration within the ecology of oral environment as well as supports saprophytic environment formation where both encourage the growth of certain oral micro-organisms as *Candida albicans*.⁴² Moreover, this enhancement for biofilm formation and its adhesion onto the denture base could be main factor for inflammation of oral mucosa especially in bad oral hygiene, elderly people or immune-deficiency patients and in cases with high carbohydrates intake.⁴³

Denture stomatitis is a common problem among acrylic complete denture wearers, mutual association occurs with *Candida* species that appears as homogeneous erythema affecting the palatal mucosa.¹⁵ Because of the development of several antibiotic resistant micro organisms, the use of antiseptics containing silver as their base is increasing in popularity as they are able to guarantee antibacterial activity in low concentrations and are less prone to cause microorganisms to acquire resistance to them than antibiotics.³⁵ Many studies evaluated the addition of AgNPs on the antibacterial and antifungal properties of acrylic resins denture base and lining material.^{23,30} They recommended that silver nanoparticles are good antifungal and antibacterial agents and may be used as alternative to the commonly used antibacterial and antifungal drugs enhancing the quality of life of the

dentures wearer.^{24,44} besides it can be used in implant associated infections.⁴⁴

After the incubation of samples of both groups for 24 hour at 37°C samples reinforced with silver nanoparticles (group II) decreases *Candida* growth than control samples (group I) as reported by Acosta-Torres⁴⁵ and Monteiro.³⁹ In another study the inhibitory effect of silver nanoparticles on *Candida albicans* was evident after 24 and 48 hour showing high effect.^{18,44} Increasing the incubation period of the samples for more than 24 hours lead to massive increase in the *Candida* colony growth thus makes it too difficult to count. Thus in-vivo testing is more recommended than in-vitro testing to make counting of *Candida* more easy and reliable as recommended.^{39,44}

In this study, the addition of 0.1% of silver nanoparticles of monomer volume gave positive results with both fracture resistance of the maxillary dentures and the antimicrobial effect against *Candida albicans*. This percentage is economically not expensive, esthetically doesn't affect the colour of the denture base moreover it will be non-toxic as it is added in nano-scale. Thus it is recommended to undergo further in-vivo studies to clarify the exact mechanism of action of silver nanoparticles and its long term effect.

Conclusion

Based on the results obtained in this study and within its limitations, it could be concluded that the addition of 0.1% of silver nanoparticles of monomer volume of acrylic resin denture bases increases the resistance of maxillary complete dentures to fracture and decreases the *Candida albicans* growth.

Most of the researches on silver nanoparticles reinforcement were done as in vitro studies. It is recommended based on the present study results, to evaluate its clinical applications especially in low concentration like 0.1% of acrylic monomer volume so as it can be widely used in dental practice as an alternative

treatment option to improve patient's oral health.

Declarations

Funding of the Study

This study is self-funded. It did not receive any financial support.

Availability of data

The data collected and analyzed in the present study are available by the corresponding author.

Ethical approval

The present study is an in vitro study

Competing interests

The authors declare that there is no conflict of interest.

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