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Effect of Adding Different Nano Particles on Antimicrobial Properties of Heat Cured Acrylic Resin

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ABSTRACT

Aim: This study was performed to examine the anti-microbial effect of different percentages of titanium dioxide and chitosan nanoparticles to heat cured poly methyl meth acrylate .**Subjects and methods:** 120 specimens of heat cure acrylic resin were grouped according to nano additives concentrations for testing anti-microbial effect. **Results:** the addition of chitosan nano particles to PMMA in 0.3% and 0.5% decreased the microbial biofilm formation in comparison to the control (PMMA without chitosan). the addition of Tio2 nano particles in concentrations 1% and 2% decreased the microbial biofilm formation more than the chitosan nano particles concentrations 0.3 and 0.5. **Conclusion:** The addition of chitosan nano particles to PMMA in 0.3% and 0.5% decreased the microbial biofilm formation in comparison to the control (PMMA without chitosan). the addition of Tio2 nano particles in concentrations 1% and 2% decreased the microbial biofilm formation more than the chitosan nano particles. The higher concentrations of the chitosan and Tio2 nano particles resulted in stronger antimicrobial effect.

KEYWORDS

nano particles, antimicrobial properties, titanium dioxide, chitosan nanoparticles, heat cured poly methyl meth acrylate.

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INTRODUCTION

Many patients suffer from physiological and functional disorders as a result of teeth loos, thus satisfactory prosthesis is indicated for their rehabilitation [(1)].

Acrylic resin polymethyl methacrylate (PMMA) has been the most popular material for the denture construction for several decades as it has many advantages as good aesthetics, perfect fit, biostability, easy laboratory and clinical management, and is considered an economic material [(2)].

Nevertheless, this material has shown some drawbacks in dental and prosthetic practices. Regarding the antimicrobial effect, this material exhibits great porosities as well as surface roughness which enhance the colonization of microbes to PMMA surface [(3,4)]

Accumulation of microorganisms on the surface of acrylic resins is one of the most challenging issues in the use of these materials. The accumulation of these microorganisms increases the incidence of oral diseases and dental caries[5]. Decreasing the microbial action in oral cavity is an important issue in the prevention and treatment of caries and in restorative dentistry and dental prosthetics. Recently, researchers' attention has shifted toward integration of NPS to acrylic resin as a preventive method which does not depend on patient cooperation to enhance its properties [6].

Owing to their large surface, minute size, and high surface energy and charge density permit nanoparticles to interact with the cell membrane, easily penetrate into a pathogen cell, and cause microbial death, NPS are considered breakthrough in dental and prosthetic practice [7].

Several nanoparticles (NPs) have been used for enhancement of antimicrobial effect of dental materials. Among various types of NPs, TiO2 (metal NPs) is an FDA-approved component for drugs, cosmetics, and food packaging uses. Exhibits excellent mechanical and physical properties as well as antimicrobial action against viruses, fungi, and bacteria [8]. As mentioned before, PMMA exhibits great porosities which act as reservoir for bacterial colonization which cause oral disease. So, some findings have shown the effect of addition of TiO2 to PMMA has greatly decreased the denture base porosity [9].

Recently, Chitosan has gained popularity in dental practice as it is biocompatible, nontoxic and biodegradable polysaccharide in addition to superior antimicrobial effect. Chitosan acts as bacterial cytotoxic material by adhering to bacterial cell wall and destroying its structure [10].

This study was performed to examine the effect of adding different percentages of titanium dioxide and chitosan nanoparticles to heat cured poly methyl meth acrylate in regard to the anti-microbial effect.

MATERIALS AND METHODS

An in vitro study was directed to evaluate the effect of adding chitosan nanoparticles with concentrations (0.3% and 0.5), Titanium di oxide nanoparticles concentrations (1% and 2%) by weight to the poly methyl meth acrylate acrylic polymer (PMMA) on its anti-microbial properties.

A total 120 rectangular bar shaped specimens were used for antimicrobial testing.

Table (1) *Materials used in the study.*

Material	Specification	Manufacturer
Heat cured acrylic	PMMA heat cured acrylic resin	Paseo de La Estacion (Madrid, Spain)
Chitosan	**Chitosan nano particles**	Nano-gate Company, Egypt
Tio2	**Titanium di oxide nano particles**	Nano-gate Company, Egypt

Preparation of PMMA + nano particles:

The powder of chitosan nano particles was mixed with (PMMA) powder at two concentrations 0.3 and 0.5w/w, of PMMA by meaning of ball milling for 2h to get homogenous mix, for (0.3% 0.3gm) chitosan nano particles was mixed with 99.7 gm typically as well for 0.5%, 0.5 gm. of chitosan nano particles mixed with 99.5 gm. TiO2 nano particles two concentrations (1% and 2%) were mixed to the (PMMA) by the same technique.

Sample preparation:

The mold was made by wax pattern technique. The processing of samples was performed according to manufacturer instructions (3:1 by volume) till reaching the dough stage. Packing in the flask then curing for 1.5 hours at 74˚C, then boiling for 1h. After polymerization, the flasks were cooled slowly at room temperature for 30 minutes and then exposed to running water for 15 minutes before removing the samples from the flasks. Then samples were dried, finished and polished. The specimens were inspected visually for any defects to discard the defective specimens.

Sample grouping:

Table (2) *120 heat cured PMMA specimens were prepared and distributed according to the following table:*

Microorganisms	Negative Control	Positive control	Samples treated with			
			PMMA with Chitosan 0.3%	PMMA with Chitosan 0.5%	PMMA with Titanium di oxide 1%	PMMA with Titanium di oxide 2%
Candida albicans	10	10	10	10	10	10
Streptococcus mutans	10	10	10	10	10	10

Negative control: PMMA without colonization
Positive control: PMMA with colonization

Test for biofilm formation

Tube adherence method recommended by **Neopane *et al.* (2018)** [11,12] was applied with some modifications. The bacterial strain (*Streptococcus mutans*) was inoculated in 3 mL nutrient broth (NB) in test tubes and incubated overnight at 37°C along with the samples. The medium contained (g/L): yeast extract, 2; peptone, 5 and NaCl, 5 (12) (**Razdan *et al*., 2019**) [13].

The same technique was also used for the yeast like fungus *Candida albicans*. This organism was cultured in tubes containing Sabouraud`s dextrose broth (SDB) which contained (g/L): peptone, 10 and dextrose, 40. Chloramphenicol 0.5 g/L was added to check bacterial growth (**Pitt and Hocking 2009**)[14].

After incubation, the tubes were decanted, dried and stained with 0.1% Crystal Violet (CV) in case of bacteria or Lactophenol cotton blue (LCB) in case of fungi. Subsequently, the tubes were washed gently and placed upside down for drying. Visible lining of the wall and bottom of the tubes and on samples by a microbial film was considered as positive. The results were scored visually as non-producers, or weak, moderate or strong biofilm producers [13] (**Manandhar *et al*., 2018**)[15].

Microscopic examination and imaging of *C. albicans* and *S. mutans* were performed using Axiostar trinocular microscope, made by Zeiss, Germany. The microscope is provided with a digital camera Canon G6, 7.1 megapixels, Made in Japan. All microscopic images were taken at X1000 magnification.

RESULTS

As shown in table (3) the microbial biofilm was markedly formed on the control segments after staining with crystal violet in case of *S. mutans* or Lactophenol cotton blue in case of *C. albicans* (Figure 1). Segments treated with either chitosan or titanium di oxide exhibited lighter coloration indicating their ability to reduce (at low concentration) or prevent (at higher concentration) microbial biofilm formation.

Test tubes from which the culture medium and segments of PMMA were removed showed variable degrees of biofilm production (Table, 2 and Figure, 2). Microscopic images are illustrated in Figure (3).

Table (3) *Biofilm production by C. albicans and S. mutans on PMMA segments using liquid culture media*

Microorganisms	Negative control	Positive control	Samples treated with			
			PMMA with Chitosan 0.3%	PMMA with Chitosan 0.5%	PMMA with Titanium di oxide 1%	PMMA with Titanium di oxide 2%
Candida albicans	-	++++	+++	++	+	-
Streptococcus mutans	-	+++	+++	+	+	-

++++: Strong, +++: Moderate, ++: weak, +: weak and -: non-producers

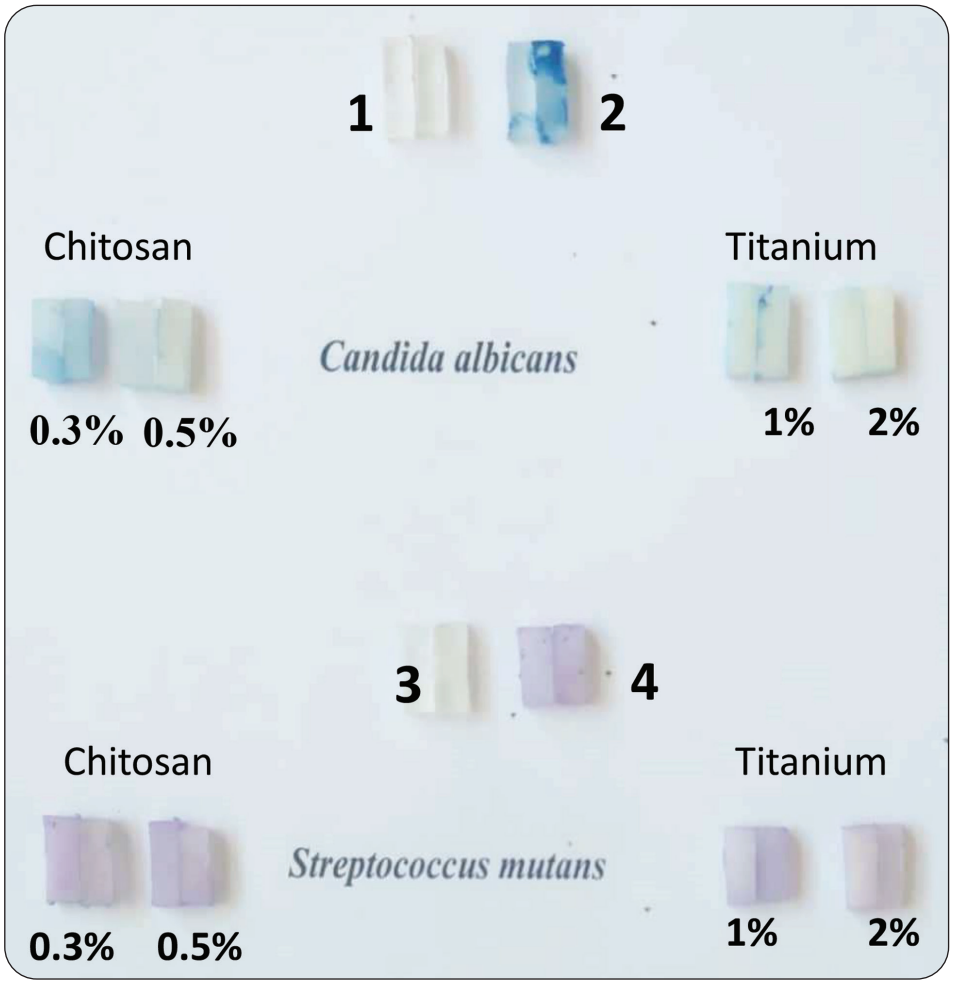

Fig. (1) Microbial biofilm formation on PMMA segments; negative

Figure (1) showing control (1 &3), high growth of *C. albicans* on control segments stained with LCB (2), moderate to weak biofilm on segments treated with PMMA with 0.3% chitosan nano particles and 0.5%, weak and no biofilm in case of PMMA with titanium dioxide nano particles. Moderate biofilm production by *S. mutans* on control segments stained with CV (4), weak biofilm production on segments treated with chitosan nano particles, weak or no biofilm in case of titanium nano particles.

Table (4) *Biofilm production by C. albicans and S. mutans on inner walls of test tubes using liquid culture media*

Microorganisms	Negative control	Positive control	Tubes containing samples treated with			
			PMMA with Chitosan 0.3%	PMMA with Chitosan 0.5%	PMMA with Titanium di oxide 1%	PMMA with Titanium di oxide 2%
Candida albicans	-	++++	+++	++	++	++
Streptococcus mutans	-	++++	+++	++	++	+

++++: Strong, +++: Moderate, ++ & +: weak and - : No biofilm production.

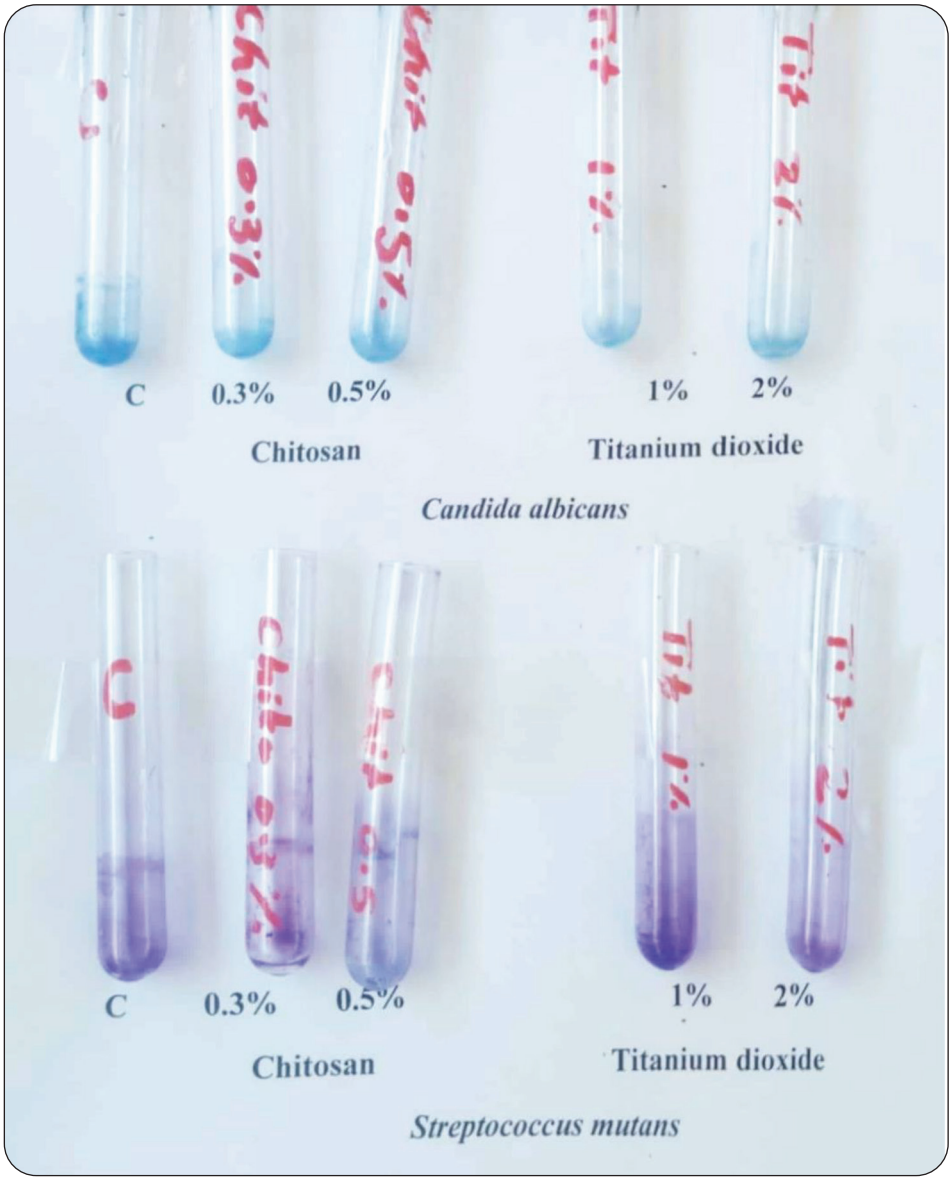

Fig. (2) Varying densities of microbial biofilm formation on the inner walls of test tubes in which untreated segments (C) negative control, high growth of C. albicans on control segments stained with LCB, moderate to weak biofilm on segments treated with chitosan nano particles 0.3% and 0.5%, weak and no biofilm in case of titanium dioxide nano particles 1% and 2%. Moderate to weak biofilm production by S. mutans on control segments stained with CV and segments treated with chitosan 0.3% and 0.5%, weak and no biofilm in case of titanium titanium dioxide nano particles 1% and 2%.

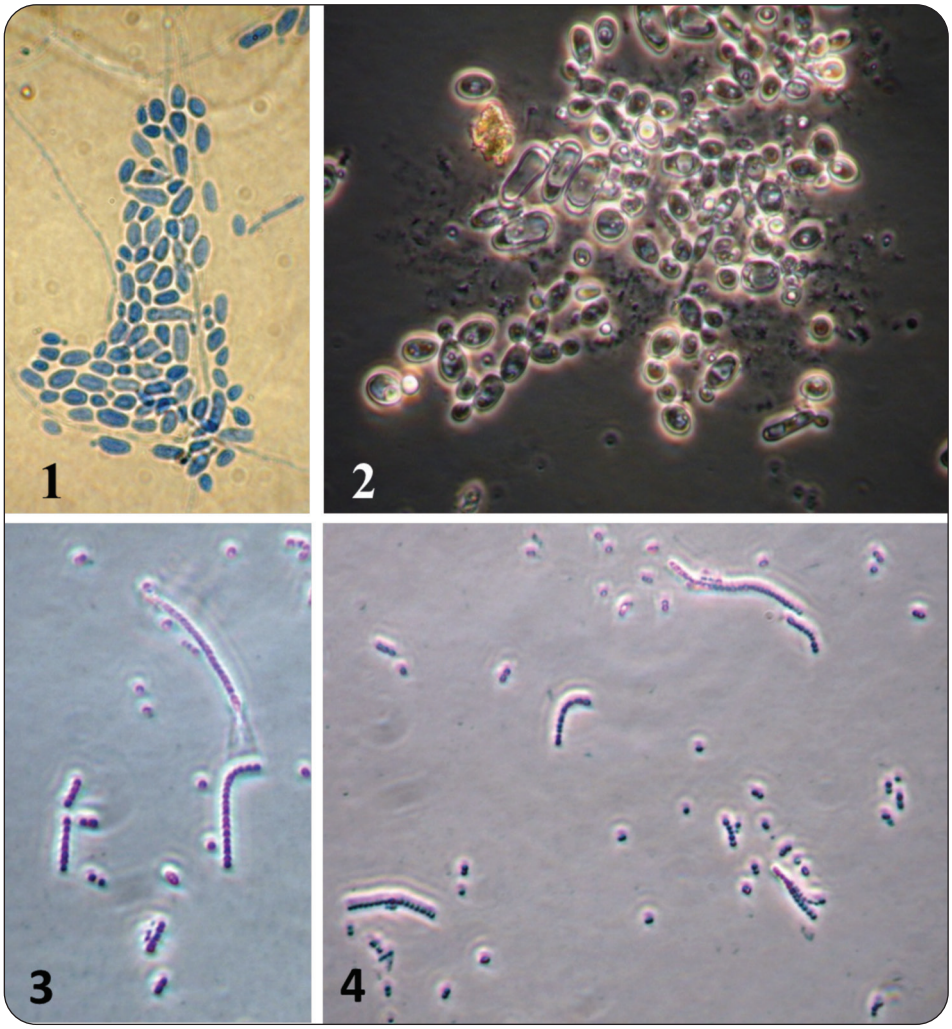

Fig. (3) Budding cells and pseudohyphae of *Candida albicans* (1, 2) and chains of coccoid shaped cells of *Streptococcus mutans* (3, 4) (Magnification X1000).

DISCUSSION

Many studies informed an increased anti-microbial outcome, regardless of the particle size of TiO2 nano particles, but dissimilar effects were noted on increasing the amount of the added TiO2 nano particles. **Cascione e**t al. found a decrease in the Candida colonization by 19% on adding of 3% TiO2 nano particles compared to the 16% reduction observed with 1% TiO2 nano particles. Opposing to this, despite showing antimicrobial effect, **Giti** and others found no substantial rise in the antimicrobial properties with rise in the nanoparticles percentage from 2.5% to 7.5%.53 likewise, **Kaurani** and others found better results on using 0.3% of TiO2 nano particles compared to 0.4% and 0.5%. [16,17,18].

Methods for testing properties:

Significant differences in the methods used in testing were recorded in which researchers used procedures as antibacterial adhesion test, minimum inhibitory concentrations (MIC) of the NPs using microbroth dilution method and biofilm formation, MIC by broth culture, the plate count method were used.45,47,51,53,55,57,58[19,20,21].

Bacteria and yeast are commensally present in healthy oral cavity both in dentulous and edentulous patients. Some situations may increase the number of these microorganisms causing colonization transferring them to be pathogenic, among these situations are denture wearing and poor denture care and hygiene [22,23].

In the existent study, The outcomes displayed that the adding of TiO2 NPs 1% and 2% to the heat cured PMMA had a wide-ranging effect on decreasing the colonization of S. aureus, and C. albicans than the control heat cured PMMA. this result compatible with **Nourhan** et al and **Alrahlah** et al, who confirmed the practical significance of the addition of TiO2 NPs [24].

Furthermore, the outcomes of current study were similar to **Natarajan** et al who discovered that TiO2 has been used widely for destroying different species of microorganisms including bacteria, fungi and viruses[25].

Chitosan has been recognized as an antimicrobial agent, however its ability to act in this way is not completely elucidated as several different mechanisms have been attributed to this nature of chitosan[26,27]. One theory suggests that when exposed to bacterial cell wall, chitosan promotes displacement of Ca++ of an ionic site of the membrane, resulting in cellular destruction [28].

When the acrylic resin mixed with chitosan nano particles, the S. mutans growth decreased with the increasing concentration of chitosan. Chitosan's antibacterial effect is thought to be related to its binding to the negatively charged bacterial cell wall, thereby causing cell breakdown and affecting membrane permeability; in addition, chitosan binds to the bacterial deoxyribonucleic acid (DNA), thus inhibiting DNA replication and ultimately leading to cell death [29].

Results of the present study showed that the antimicrobial effect increased by increasing the percentage of the added chitosan nanoparticles to the heat cured acrylic resin, these results were in accordance with [30,31]. But increasing the percentage of chitosan can affect the mechanical properties of the heat cured acrylic adversely, for this reason its recommended to add chitosan nano particles with a percentage less than 1% [32].

CONCLUSIONS

Within the limitations of this study, the addition of chitosan nano particles to PMMA in 0.3% and 0.5% decreased the microbial biofilm formation of *Candida albicans and Streptococcus mutans*.

In comparison to the control (PMMA without chitosan). the addition of Tio2 nano particles in concentrations 1% and 2% decreased the microbial biofilm formation of Candida albicans and Streptococcus mutans more than the chitosan nano particles concentrations 0.3 and 0.5.

The higher concentrations of the chitosan and Tio2 nano particles resulted in stronger antimicrobial effect.

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النشر الرسمي لكلية طب الأسنان
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تأثير إضافة جزيئات نانوية مختلفة على الخواص المضادة للميكروبات لراتنج الأكريليك المعالج بالحرارة

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الملخص :

الهدف: هذه الدراسة تمت لفحص خواص مقاومة الميكروبات للأكريل الحراري بعد إضافة نسب مختلفة من جزيئات النانو من مواد، اوكسيد التيتانيوم، والشيتوسان.

المواد والطريقة: تم استخدام 120 عينة من الأكريل الحرارى مقسمة لمجموعات، حسب نسب جزيئات النانو المضافة، لدراسة تأثير هذه النسب على مقاومة الميكروبات.

النتائج: ثبت من الدراسة ان إضافة 0.3% AND 0.5% من النانو شيتوسان، قلل تكوين البيوفيلم الخاص بالميكروبات على عينات الأكريل الحراري. وان إضافة 1% AND 2% من نانو أوكسيد التيتانيوم قلل تكوين البيوفيلم الخاص بالميكروبات بنسبة أعلى من الكنترول وأعلى من العينات المضاف اليها النانو شيتوسان بنسب 0.3% AND 0.5%

الخلاصة:إضافة النانو شيتوسان بنسب 0.3% AND 0.5% قلل البيوفيلم الميكروبى مقارنة بالكنترول (عينات الاكريل الحراري بلا إضافات) وإضافة 1% AND 2% من نانو أوكسيد التيتانيوم لعينات الاكريل الحراري قلل تكوين البيوفيلم الميكروبي أكثر من عينات الكنترول وعينات النانو شيتوسانو ثبت ان زيادة تركيز جزيئات النانو من المادتين زود مقاومة الأكريل الحراري لتكوين البيوفيلم الميكروبي.

الكلمات المفتاحية: جزيئات النانو، خصائص مضادة للميكروبات، ثاني أكسيد التيتانيوم، جزيئات الشيتوزان النانوية، بولي ميثيل ميث أكريلات المعالج بالحرارة