



RESEARCH ARTICLE - MEDICAL TECHNIQUES

Effect of Aging on the Antifungal Activity and Surface Roughness of Soft Lining Material Incorporated with Chitosan Nano-Particles

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Article Info.	Abstract
<p><i>Article history:</i></p> <p>Received 23 January 2022</p> <p>Accepted 19 February 2022</p> <p>Publishing 31 March 2022</p>	<p>Background: Chitosan is a natural bio-polymer, which has been utilized in different pharmaceutical and biomedical fields, and it has antimicrobial activities. Resilient liner, it's a polymeric material, considered as a kind of "shock absorber".</p> <p>Purpose: The purpose of this study is to evaluate the effects of immersion in the artificial saliva on the antifungal activity and surface roughness of heat-polymerized soft lining material impregnated with CHs-NPs.</p> <p>Approach: 180 specimens were prepared. Divided into 3 groups according to the test: Sensitivity test, viable count test and surface roughness test. Then the specimens subdivided into 3 groups depending on the Chitosan addition and the different pH of saliva. The sample's dimensions for candida viability count test were (10×10×2.3mm) length, width, and thickness, while a plastic disc with dimensions (6 diameter ×0.5mm thickness) for disc diffusion test. For surface roughness test a disc with 30mm diameter and 3mm thickness. The data analyzed statistically using SPSS, (ANOVA) test with Games-Howell test to accept or reject the statistical hypothesis.</p> <p>Results: For viable count test, there was a significant decrease in CFU/ml of <i>C. albicans</i> after incorporation 3.5% CHs-NPs, the results also showed that the specimens' group that was stored in acidic artificial saliva, recorded a significant increase in CFU/ml of <i>C. albicans</i>, followed by the specimens' group that was stored in alkaline artificial saliva. While for disk diffusion test, results showed there was no inhibition zone around any PEMA disks except the experimental group that was stored in neutral artificial saliva. The results of surface roughness, showed no significant difference after incorporation in 3.5% of CHs-NPs into the soft denture liner and immersion in the artificial saliva with different pH levels.</p> <p>Conclusion: Acidic and alkaline artificial saliva encouraging the growth <i>C. albicans</i>, while the neutral artificial saliva had the least effect. Also, the result of the study concluded that vertex soft lining material impregnated with chitosan nano-particles aids in obtaining a lining material with the antifungal activity against <i>C. albicans</i>. While the aging process and the addition of CHs-NPs was not having a significantly effect on the surface roughness.</p>

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Keywords: Soft Lining Material; Chitosan Nano-Particles; Viable Count Test; Disk Diffusion Test; Surface Roughness; Complete Denture.

1. Introduction

For more than one century, soft denture lining materials have been utilized in dentistry field [1]. A soft denture lining material also known as a resilient liner, it's a polymeric material, considered as a kind of "shock absorber" between the occluded surfaces of the denture and the underlying oral mucosa to assimilate the mastication forces and distribution it by the means of viscoelastic behavior [2]. There are several conditions needed to use the flexible lining materials for complete dentures like patient has irregularities in the alveolar margin and covered with relatively thin, non-pliable mucosal layer, this is could be painful when chewing forces applied through a solid acrylic bases on this kind of supportive tissue so utilizing soft lining materials will aid in relieving the pain and increasing patient acceptance of dentures [3, 4]. Around the worldwide, the fungal infections are showing an increasing prevalence. Candida species have many ferocity factors that promote the colonization, and by appropriate conditions the candida spp. have the opportunistic of infection of host organisms. *C. albicans* is considered the most predominant isolate from oral mucosal infections among all the candida species, e.g., oral candidiasis and denture stomatitis [5]. For relined denture, *C. albicans* firstly adheres to the lining surface then it penetrates inside the material and this adherence is the first step in colonization and the advancement of pathogenesis [6].

Nomenclature & Symbols			
AS	Artificial Saliva	CHs-NPs	Chitosan Nanoparticles
G	Gram	PEMA	Poly-Ethyl Methacrylate
S	Significant	mm	Millimeter
SPSS	Statistical Package for Social Science	SDA	Sabouraud Dextrose Agar
P	P-Value	SDB	Sabouraud Dextrose Broth

Also, the surface roughness of the lining material is considered most significant issue, cause it's directly or indirectly, encourages the adhesion of microorganisms and promoting the colonization, proliferation of fungal and bacterial species, staining, and plaque accumulation thus it affects the health of denture-bearing area and patient's comfort that were compromised the longevity of the material [7]. So, the ideal softness of lining materials is very necessary and must be remained constant for a while, whether the material is permanent or temporary so that the material can do their job efficiently [8].

For avoiding these drawbacks, Chitosan nano particles was impregnated within the lining material [9]. Chitosan is a natural bio-polymer, derived from the outer shell of shrimp and some kind of insects and characterized by its biocompatibility. Due to it has safe profile, it was utilized in different pharmaceutical and biomedical fields, also it has bacteriostatic and fungi-static action [10]. Chitosan nanoparticles have the properties and characteristic of both chitosan as a natural polymer and nanoparticle [11]. On the other hand, in the oral cavity denture lining materials functions are sensitive to salivary pH [12]. During the day, the pH of human saliva differs in response to various factors like ingestion of some foods and some clinical conditions can cause changing in salivary pH from the value that is neutral to less acidic value [13]. Cheese, fish, meat, cereals, orange juice, dessert and pastries, tend to make the salivary pH more acidic also smoking and some diseases like chemotherapy and Sjögren's syndrome, while some food like vegetable and fruits is considered alkaline-forming foods also some digestive problems such as enzyme production and pancreas secretions tend to make the salivary pH more alkaline [14].

Several studies have been conducted on the effect of water, different beverages and denture cleansers on the properties of soft lining materials [4]. But there are no articles published regarding the effect of artificial saliva with different pH levels on the antifungal activity of soft lining materials. Therefore, the current study was designed to assess the effect of artificial saliva with different pH degrees on the antifungal activity and surface roughness of heat-cured acrylic based soft denture lining material impregnated with CHs nano-particles.

2. Materials and Method

2.1. Preparation of artificial saliva

First of all, the artificial saliva was prepared in three different pH levels neutral, acidic and alkaline. The composition of electrolytic salts that were utilized in preparation of the artificial saliva, very similar to human saliva including: 0.260 g/l of Na_2HPO_4 , 0.700 g/l of NaCl , 0.330 g/l of KSCN , 0.200 g/l of KH_2PO_4 , 1.500 g/l of NaHCO_3 and 1.200 g/l of KCl . Also, 0.2 g urea ($\text{CH}_4\text{N}_2\text{O}$) and 2.7 g of mucin were added [15]. Preparation of buffer solution by dissolving each of KH_2PO_4 and Na_2HPO_4 salts in 1 liter of boiled de-ionized distilled water. Preparation of acidic solution (5.7 ± 0.01) was done by placing 500 mL of KH_2PO_4 in gradient flask and gradually added Na_2HPO_4 solution till the exacted level of pH was reached, then the remaining salts were added and the volume was finished to 1liter by de-ionized distilled water. As previous manner, the neutral artificial saliva solution was prepared but the amount of disodium phosphate (Na_2HPO_4) which was added to the solution was greater and it was added gradually and checked with pH meter till the required level of pH (7) was obtained, while for preparation of basic artificial saliva (8.3 ± 0.01) 500ml of Na_2HPO_4 was firstly placed in gradient flask and gradually added KH_2PO_4 solution till the required level of pH was reached, then the remaining salts were added and the volume was finished to 1 liter by de-ionized distilled water [14].

2.2. Specimens' grouping

180 specimens were prepared in this study. The specimens were divided into three groups according to the test to be performed: Sensitivity test, viable count test and surface roughness test. For each test, 60 specimens were prepared, then the specimens will be subdivided into 3 groups depending on the Chitosan addition and the different pH of saliva storage media: First group: 20 specimens were stored in Neutral media; 10 specimens were prepared with addition of CHs-NPs and other 10 were made without addition as control group. Second group: 20 specimens were left in an Acidic media; 10 specimens were prepared with addition of CHs-NPs and other 10 were made without addition as control group. Third group: 20 specimens were left in an Alkaline media; 10 specimens were prepared with addition of CHs-NPs and other 10 was made without addition as control group.

2.3. Fabrication of CHs-NPs loaded soft liner specimens

2.3.1. Specimen's design

1. The dimensions of the sample for candida viability count test were (10×10×2.3 mm) length, width, and thickness respectively [16], while a plastic disc with dimensions (6×0.5 mm) diameter and thickness respectively were utilized for disc diffusion test [17].
2. According to ASTM (D. 2240-00 2002), Specimens' dimensions of surface roughness test was a disc shape with 30 mm diameter and 3 mm thickness [18].

2.3.2. Proportioning, mixing ratio and incorporation of CHs-NPs with soft liner powder

To weigh chitosan nanoparticles powder and the powder soft denture lining material, precision electronic balance was used. An amalgamator device used for mixing chitosan nanoparticles powder with fine liner powder for 1 minute with a mixing frequency 3000 vibrations /minute to ensure uniform and homogenizes distribution of nanoparticles inside the polymers.

After that, per manufacturer's directions the specimens were prepared (P/L ratio: for each 1ml of liquid monomer /1.2g of powder). 45 gm of soft-liner powder was weighed for both groups and mixed with 37.5 ml of monomer, according to the following equation [9].

$$\frac{1 \cdot 2 \text{ gm}}{45 \text{ gm}} = \frac{1 \text{ ml}}{x} \quad \longrightarrow \quad x = \frac{45 \text{ gm} \times 1 \text{ ml}}{1 \cdot 2 \text{ gm}} \quad \longrightarrow \quad x = 37.5 \text{ ml}$$

In clean glass container, the mixing was done and taking into account that the weight of CHs-NPs powder was subtracted from the total weight of soft-liner powder to get the accurate P/L ratio.

2.3.3. Packing, curing and finishing

When the mixture reached the dough stage, it was loaded into the mould and covered with polyethylene sheets, the two halves of the flask were assembled and placed under the hydraulic press with continuous application of pressure (100Kg/cm²) for about five minutes to provide even flowing the material throughout the mold space, then removed the pressure and the two halves of the flask were opened, removed the polyethylene sheets, then with the using of sharp knife, the excess material was cut, then applied another layer of separating medium and left aside to dry. Finally, reassembled the two halves of the flask and place under the press for establishing metal to metal contact, then clamping was done and transferred to water bath for curing [19]. The curing process was done according to the manufacturer directions of lining Material (heating up to 70°C for 90 minutes then the temperature was raised up to 100°C and kept for 30 minutes). When the curing process ended, the clamped removed from the water bath is removed from the water bath and left to cool gradually to room temperature for 30 minutes, then cooling it under running tap water for 15 minutes. Then de-flasking and all the specimens were finished by utilizing sharp blades and polished with fine grit silicon polishing bur and fine grit sand paper, after that all the specimens were autoclaved for sterilization.

2.4. Microbiological part of the study

First of all, sabouraud dextrose agar was prepared and autoclaved following the instructions that supplied by the manufacturer. After that mixed with 0.05g of chloramphenicol to inhibit wide range of bacterial growth [20]. Then the isolation medium was poured into sterilize petri-dishes and left it, to cool down to room temperature, then kept in the refrigerator at 4°C for being used later. *C. albicans* was isolated from patients mouth with symptoms of denture stomatitis by direct technique which involves gently touching a sterilized cotton swab over the lesion site then inoculated the isolation medium then aerobically incubation was done at 37 °C for (24-48 hours), finally, it saved in cool place (4°C) to be used later [21]. After that, identification was done following different approaches.

2.4.1. Microscopic examination

This test was done by utilizing an inoculation loop, taking isolated colony and then on a glass slide, and the colony was emulsified in a drop of normal saline for forming candidal suspension, then with wood stick the suspension was spread on the slide and allowed to dry to room temperature, passed several times over the burner flame for fixation, and the slide was stained following the directions of Gram's method by utilizing the stain kit [22]. Finally, the slide was examined under light microscope and showing spherical budding yeast, look like cells [23].

2.4.2. Formation of germ tube

This test was performed by taking pure, an isolated colony, then the colony was suspended in 0.5 ml of human serum to stimulate the growth of pseudo-hyphae after it had been incubated at 37 °C for two hours [24].

2.4.3. Biochemical Identification

Biochemical identification The VITEK 2 YST system is a fully automated device with sensitive technology, based on enzyme detection. Prior to testing, an applicator rod was used to take sufficient number of pure, isolated colonies and suspend it in 3.0 mL sterilize saline polystyrene in a clear test tube and then according to McFarland standard, the turbidity was checked. The VITEK card is then automatically filled with the prepared candidal suspension, closed and incubated at 35.5°C for 18 hours. At last, the outcomes were compared with the database and unknown microorganisms were identified [7].

2.5. Assessing the effect of CHs-NPs / soft liner samples on viable count of *C. albicans*

60 samples with dimensions (10 × 10 × 2.3 mm) width, length and thickness, respectively were prepared for viable count test [16]. After that all the specimens were autoclaved and then subjected to the storage protocol, 20 specimens were stored in neutral artificial saliva (10 without any addition which was considered, the control group and 10 with addition), 20 specimens were stored in acidic artificial saliva and 20 specimens were stored in alkaline artificial saliva.

For simulation of the patient's daily usage of the dentures, all the samples were subjected to the storage protocol, and this was done by storing it for 16 hrs. in artificial saliva in the incubator at 37 °C and 8 hrs. inside the distilled for 30 days. As the pH of the artificial saliva changed within 48 hrs., it was changed every day days [25].

According to the manufacturer's directions, Sabouraud dextrose broth were prepared, autoclaved at 121 °C/15 psi for 15 min and stored. Then, pure, single colony of fresh cultured *C. albicans* was taken and diluted in 0.9% NaCl to form yeast suspension of about 107 CFU/ml (0.5 McFarland standards) and examined the result with a McFarland densitometry. Each specimen was placed into sterile tubes containing 9.9 ml of previously prepared SDB and 100µl of yeast suspension and then incubated for 1 day at 37 °C. After incubation, all samples were taken, removed from the suspension and rinsed five times in sterile deionized water to eliminate loosely adherent cells [26]. Viable cells were propagated into SDA plates and then counted and statistically analyzed after incubation according to the following equation.

$$AF [\%] = (V_c - V_t) / V_c \times 100\%$$

The number of viable fungal colonies of the control samples was V_c and V_t was the number of viable fungal colonies of the experimental samples [16].

2.6. Assessing the effect of CHs-NPs / soft liner samples by using (Kirby- Baure) disk diffusion test

60 CHs-NPs/ soft lining discs with dimensions (6×0.5 mm) diameter and thickness respectively were utilize for disc diffusion test [17]. After that all the specimens were autoclaved and then subjected to the same storage protocol like the specimens of viable count test.

Following the guidelines of CLSI 2016, Mueller-Hinton agar was prepared, autoclaved and poured in sterilized petri-dish plate. Then with the using of inoculation loop, a pure and single colony of freshly cultured *C. albicans* was taken and diluted in sterile 0.85% saline to form candidal suspension then using a sterile cotton swab immersed in the suspension, the swabbing was performed in three directions to obtain even growth of *C. albicans* on the agar surface and left for 5 min. after that the specimens were mounted on the agar surface afterwards all plates were left at room temperature for two hours and then incubated aerobically at 37°C for 24 h. and then using a digital caliper ruler, to measure the area of inhibition that appeared [27].

2.7. Roughness Measurement

For measuring of the surface roughness of soft lining samples, (Portable surface roughness cages, Mahr federal Inc, Germany), with ($0.001\mu\text{m}$) accuracy was utilized. This device is equipped with surface analyzer which was conical diamond stylus for tracing of the surface irregularities. On the same surface of the specimen, three readings were recorded, one reading was recorded in the middle while the other two readings were recorded on each right and left side, the reading appeared on the digital display and it was measured in Ra parameter which means as the arithmetic mean of surface roughness (ASME B46.1, 2009). The average of the three readings was considered, the roughness testing value.

3. Statistical Analysis

The data analyzed statistically using software computer program (SPSS) to performed the descriptive statistics and the inferential statistics including analysis of variations (ANOVA) test with Games-Howell test to accept or reject the statistical hypothesis.

4. Results

Firstly, the results of SEM analysis confirmed the size of CHs-NPs powder with the average was nearly ≥ 50 nm. After that, SEM results of soft liner before (control) and after (experimental) the addition of 3.5% by weight CHs-NPs particles powder were presented in fig. 1. The result of SEM analysis for the experimental groups displayed that there were a fair distribution and some degree of the CHs-NPs agglomeration into the soft lining matrix. Also, at 1 kx magnification, the surface topography for the specimens of the control groups displayed micro-cracks and porous and they reduced to greatest extent after addition CHs-NPs.

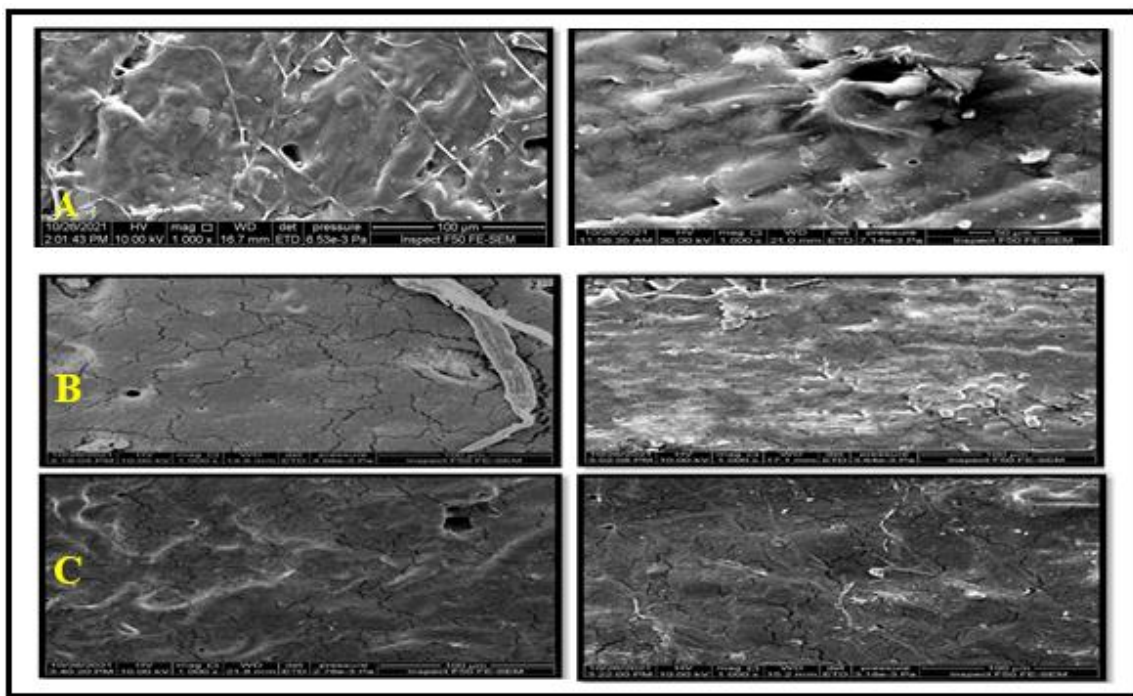


Fig 1. SEM images for soft liner samples at 1 kx magnification power; before CHs-NPs addition and after CHs-NPs addition; (A) Stored in alkaline AS, (B) Stored in neutral AS, (C) Stored in acidic AS

4.1. Count of *C. albicans* (CFU/ml)

4.1.1. The effect of different pH levels of artificial saliva and CHs-NPs addition on viable count of *C. albicans* (CFU/ml)

Generally, the viability count of *C. albicans* was reduced with addition of 3.5% CHs-NPs. According to different pH of saliva, the specimens that were stored in neutral artificial saliva recorded the maximum reduction of candida count followed by the specimens that was stored in alkaline artificial saliva, while the specimens that were stored in acidic media recorded minimum reduction of candida count.

As shown in table 1 and fig. 2. The maximum value found through the control group while the minimum value found through the experimental group.

For comparison between all groups depending on different pH levels of artificial saliva, ANOVA test table of viable count of *C. albicans* was used and revealed a significant difference among all the study groups, table 2.

Table 1 Descriptive statistics of viable count of *C. albicans* for all study groups

Groups	N	Mean	Std. Deviation	Std. Error	Min.	Max.
Control groups (Neutral)	10	58.20	7.052	2.230	43	79
Control groups (Acidic)	10	289.10	45.229	14.303	179	387
Control groups (Alkaline)	10	81.50	8.141	2.574	59	39
Experimental groups (Neutral)	10	15.20	2.530	.800	3	27
Experimental groups (Acidic)	10	163.00	19.476	6.159	136	187
Experimental groups (Alkaline)	10	24.30	5.774	1.826	11	111

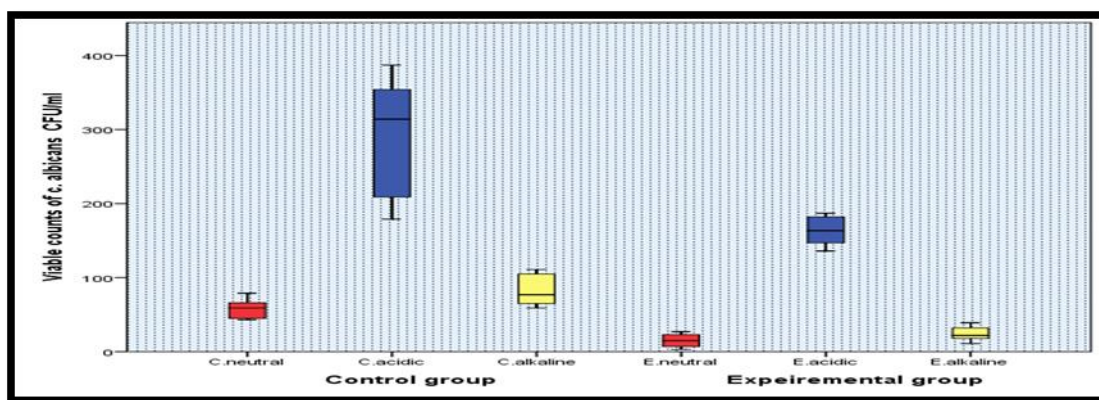


Fig 2. Boxplots of viability count for each control and experimental group

Table 2 ANOVA table of viable count of *C. albicans* for all study groups

Source of variation	Mean Square	F-test	P- value	Sig.
Between Groups	109151.097	253.767	0.000	S*
Within Groups	430.124			

* S: Significant at p-value ≤ 0.05

According to the result of the homogeneity of Levene's test for equality of variances data, table 3, which was indicated significant differences between groups of variances, so for multiple comparisons Games-Howell Post Hoc test was chosen of viable count of *C. albicans* test. The results were revealed that there was significant difference among all groups, table 4.

Table 3 Levene's test of homogeneity of viable count of *C. albicans* test

Levene Statistic	P-value	Sig.
11.591	0.000	S*

* S: Significant at p-value ≤ 0.05

Table 4 Games -Howell test of viable count of *C. albicans* test

Groups	P-value	Sig.
Control groups (Neutral * Acidic)	0.000	S*
Control groups (Neutral * Alkaline)	0.000	S*
Control groups (Acidic* Alkaline)	0.000	S*
Experimental groups (Neutral * Acidic)	0.000	S*
Experimental groups (Neutral * Alkaline)	0.006	NS**
Experimental groups (Acidic* Alkaline)	0.000	S*

* S: Significant at p-value ≤ 0.05 %, ** NS: Non-significant at p-value ≥ 0.05 %

4.1.2. The effect of CHs-NPs addition on viable count of *c. albicans*

The difference in the mean values between the control and the experimental groups of viable count of *c. albicans* test were showed significant difference between them as shown in table 5.

Table 5 Independent t-test for equality of means for viable count of *c. albicans*

Groups	df	T-value	P-value	Sig.
Control Neutral * Experimental Neutral	18	5.084	0.000	S*
Control Acidic * Experimental Acidic	18	5.084	0.000	S*
Control Alkaline * Experimental Alkaline	18	8.129	0.000	S*

* S: Significant at p-value ≤ 0.05

4.1.3. The result of Kirby- Baure disk diffusion test

The results showed that there was no inhibition zone around any PEMA disks of any control or experimental groups except the experimental group that was stored in neutral artificial saliva.

4.2. The result of surface roughness test

4.2.1. The effect of different pH levels of artificial saliva and CHs-NPs addition

Descriptive statistics of surface roughness test results for all the studied groups were presented in table 6 and fig. 3. Results showed the highest mean value among control group that was stored in alkaline artificial saliva, while the lowest mean value was for the experimental group that was stored in acidic artificial saliva. The maximum value found among the control group, while the minimum value found among the control group.

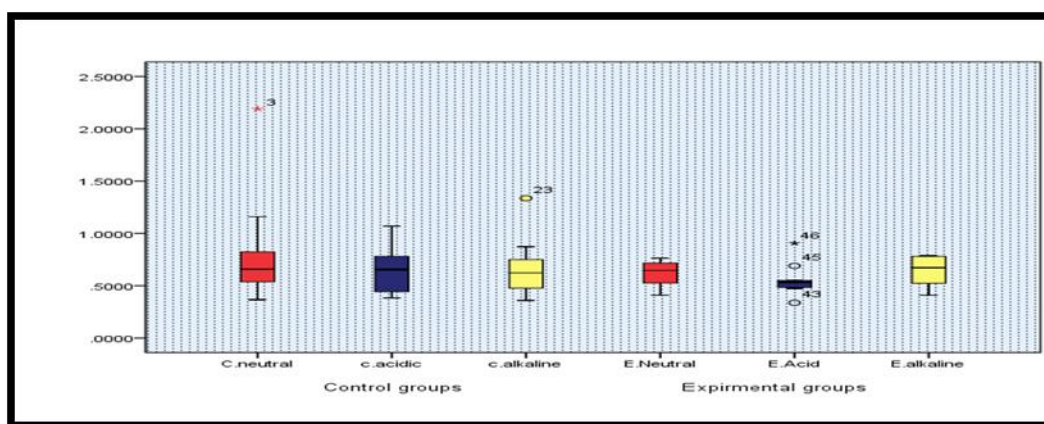


Fig 3. Boxplots showing the mean values of surface roughness test of all study groups

Table 6 Descriptive statistics of surface roughness test of the studies groups

Groups	N	Mean	Std. Deviation	Std. Error	Min.	Max.
Control groups (Neutral)	10	0.8187	0.5276	0.1668	0.3667	2.190
Control groups (Acidic)	10	0.6713	0.2417	0.0764	0.3833	1.070
Control groups (Alkaline)	10	0.6743	0.2813	0.0889	0.360	1.337
Experimental groups (Neutral)	10	0.618	0.1254	0.0397	0.410	0.763
Experimental groups (Acidic)	10	0.5567	0.1508	0.0477	0.3367	0.9067
Experimental groups (Alkaline)	10	0.6413	0.1458	0.0461	0.4100	0.7867

For comparison between all groups depending on different pH levels of artificial saliva, ANOVA test table for surface roughness revealed that there was no significant difference among all study groups (p-value > 0.05), table 7.

Table 7 ANOVA test of surface roughness test

Source of variation	Mean Square	F-test	P- value	Sig.
Between Groups	0.382	0.965	0.45	NS*
Within Groups	4.280			

* NS: Non-significant at p-value ≥ 0.05

5. Discussion

5.1. Antifungal activity

5.1.1. The effect of CHs-NPs addition on *C. albicans* growth

Firstly, viability count of *C. albicans* were studied and checked because it gives more scientific and accurate result in comparison with sensitivity method [28].

Statistically, the results of the present study, showed a significant decrease in colonies forming units/ml of *C. albicans* after incorporation 3.5% CHs-NPs into the soft denture liner which indicate the development of a composite with antifungal activity. Multiple explanations were proposed about chitosan antifungal activity.

First explanation was indicated that chitosan can penetrate the bacterial cell walls, binding with DNA and block DNA transcription and synthesis of mRNA, these would affect the development of essential proteins and enzymes [29]. The second one was indicated that chitosan positive charge binding with negatively charged components on the plasmic membrane of the fungi, this binding altering fungal surface morphology and forming an impermeable layer around it and that will prevent the intracellular transport [30].

Another explanation was indicated that the CHs-NPs not just exhibiting antifungal activity against the fungal strains however, influencing the morphology of *albicans* cells to a lesser virulent form which is the blastoconidia [30].

This result was agreed with [31] who study the adherence of *C. albicans* cells to the surface of the soft lining material impregnated with CHs-NPs and revealed a significant decrease the numbers of colonies adhered on experimental group specimens.

Also agreed with [32] who showed that the viable count test for RTV silicone elastomer material after incorporated with chitosan micro-particles with different concentrations (1.5%, 2.5%, and 3.5% by wt.) revealed into room temperature vulcanized silicone elastomer material was revealed a significance decrease in the numbers of *C. albicans* colonies adhered to experimental group specimens, and the disk diffusion test revealed a significance increase in the inhibition zone measurement as the chitosan concentrations increase.

Likewise, agreed with [33] who revealed that the incorporation of chitosan in tissue conditioning material made it effective against *C. albicans*. At the same time, the study findings disagreed with [34] who modified PMMA resins with CHs salt and revealed that CHs has no significant decreased in *C. albicans* counts.

While regarding disk diffusion test, there was some inhibition zones were distinguished around some specimens even after incubation in artificial saliva this may be explained by the result performed by [35] who study disk-diffusion test for PEMA/ ZrO₂-NPs specimens with different concentrations and the result showed there was no inhibition zone and this explained by disappear of zirconium from soft liner/Zr O₂-NPs composite.

5.1.2. Effect of different pH levels of artificial saliva on *C. albicans* growth

Statistically, the results of the present study showed that the specimens' group that was stored in acidic artificial saliva for both control and experimental groups, recorded a significant increase in the numbers of colonies forming units/ml of *C. albicans*; this may be related to the fact that the fungi generally have acidophilic nature [36], followed by the specimens' group that was stored in alkaline artificial saliva, and this may be related to the fact that *C. albicans* in alkaline environment stimulate hyphal formation and this is considered a key factor of *C. albicans* virulency [27].

The results were agreed with the results performed by [37] who proved the relationship between acidic saliva and the presence of *C. albicans*, candida species in the oral cavity, and oral candidiasis. Also, agreed with a study performed by [36] who studied the effect of different pH levels and temperature on yeast transition in *C. albicans* and proved that the low pH less than 6.5 stimulating and encouraging the growth of the yeast. [38] revealed that the growth of *C. albicans* was slightly higher at pH 5.8 more than pH 7.

5.2. Surface roughness

5.2.1. The effect of CHs-NPs addition on surface roughness

Statistically, the results of the present study, showed no significant difference after incorporation in 3.5% of CHs-NPs into the soft denture liner (p-value > 0.05); this may be due to presence a few particles within the sample surface that did not affect the surface roughness and as it's known that the surface roughness test confined and measured the irregularities on the outer surface not with the inner surface of the sample, or may be due to the fact that CHs-NPs has a very small size and well dispersion.

The findings of present study disagreed with [40] who modified PEMA resins with CHs-NPs with different concentrations and revealed that there was a high surface roughness for the experimental specimens; this related to agglomeration that occur in CHs-NPs through PEMA matrix; this agglomerated particle act as center for stress-accumulation and affect adversely mechanical properties of the polymerized materials.

Also, disagreed with [41] who studied the effect of reinforcement heat cured acrylic resin with different concentration of CHs salt; the result of the study showed significant increase in surface roughness for the study samples with 5 % CHs, while at 10% concentration the surface roughness was significantly decrease; this alteration could be related to the interferences that occur in the polymerization by chitosan particles or may CHs particles interfere the cross linking reaction and obtain less condensed polymer structures which leads to change the surface topography.

5.2.2. Effect of different pH levels of artificial saliva on surface roughness of soft lining material

Statistically, the results of the present study, showed no significant difference among all study groups as the p-value was greater than 0.05. According to [42], the kind of storage media with different pH levels and the composition of the soaked material are important factors for examining the degradation of polymeric materials.

Generally speaking, all the data's exhibited that the specimens' group that was stored in neutral and alkaline AS for both control and experimental groups recorded the highest mean value; this may be related to the fact that the basic AS contain a large number of hydroxyl ions, which responsible for accelerating the degradation process, thus the surface roughness of the immersed samples is increased, while the specimens' group that was stored in acidic AS for both control and experimental groups recorded the lowest mean value this is may be explained by the losing of some structural ions from acidic saliva made the polymer surface more softness and this findings agreed with [43] who studied the effect of different pH levels of AS on the surface roughness of different materials of acrylic denture base.

Also, pervious research evaluated the effect of beverages on surface roughness and hardness of soft lining material and concluded there was significant change in both properties and revealed that the soda group (alkaline group) recorded the highest surface roughness and this was in agreement with the result of current study [2].

While, contradict with previous study who concluded the surface roughness of acrylic resin was increased due to immersion in acidic pH of AS [44].

Also, this result disagreed with the result done by [45] who revealed that there was increased in surface roughness of acrylic denture base after immersed in acidic drink.

6. Conclusion

Within study limitation and based on the results of the current study, the conclusions can be listed:

1. Acidic and alkaline artificial saliva encouraging the growth *C. albicans*, while the neutral artificial saliva has the least effect. Also, the result of the study concluded that vertex soft lining material impregnated with chitosan nano-particles aids in obtaining a lining material with the antifungal activity against *C. albicans*.
2. The surface roughness of Vertex soft lining material was not significantly affected by the addition of CHs-NPs and the storage in different pH levels of AS.

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