The soft liner specimens, along with the aluminum step wedge, and wax plate were laid on top of a cassette (kodak medical x-ray film) and with 50 Kv irradiation, 200 mA, and exposure time of 0.1 sec at a distance of 1 m from the x-ray source. As an x-ray source, a Computed Radiographic System (CR) was used (Chest X-ray machine, Germany).

In accordance with the manufacturer's instructions, the processing was carried out using an automatic x-ray film processor (JP-33, Korea). The optical density was measure for of every aluminium step and soft liner specimens using a light transmission densitometer. Three measurements were made for each specimen, and the average value of these readings was calculated.

Surface hardness test

30 specimens were prepared of soft liner in disk shape of 30 mm. in diameter and 3 mm. in thickness for Shore a surface hardness test [28,29].

The soft liner samples' hardness was measured using a shore A durometer (Time group-TH200, China) [30]. Five durometer measurements were made for every sample, with 5 sec. contact time with every penetration, with the average taken into account as the test value.

Statistical analysis

For analysing the study's results, SPSS (Statistical Package for Social Science) computer software (version 21) was used, as well as descriptive and inferential statistics. A one-way ANOVA (analysis of variance) test was used. A "P" value of > 0.05 was considered statistically non-significant, a "P" value of 0.05 was considered significant, and a "P" value of 0.01 was considered highly significant.

RESULTS

FTIR analysis: FTIR analysis revealed that there has been only physical interaction between the acrylic soft liner and the $BaTiO_3$ NPs (Figure 1).

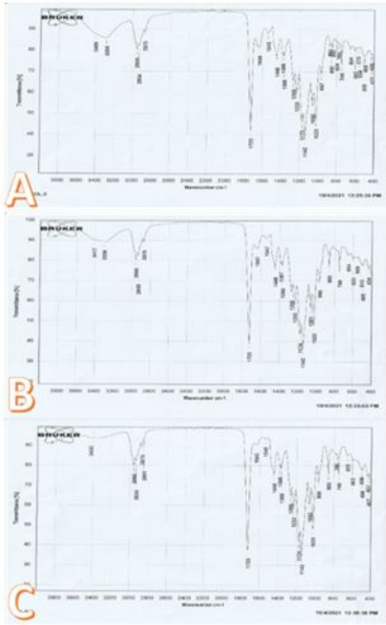

Figure 1: FTIR for A) soft liner with no addition B) soft liner with 1% by wt. $BaTiO_3$ NPs C) soft liner with 1.5% by wt. $BaTiO_3$ NPs.

Scanning electron microscopy: The morphology of cross-sectional samples and mapping of samples with soft liners before and after the addition of $BaTiO_3$ NPs are studied (Figure 2).

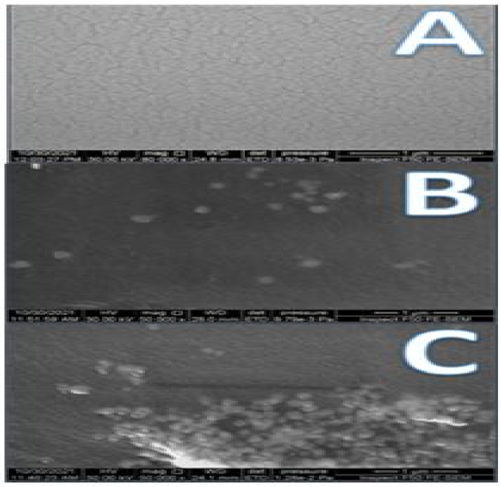

Figure 2: A) SEM image for soft liner without addition; B) SEM image for soft liner with 1% by wt. BaTiO3 NPs; C) SEM image for 1.5% by wt. BaTiO3 NPs. Energy Dispersive X-ray Spectroscopy (EDS): It indicates the existence of elements such as calcium, carbon, and oxygen in the soft liners before and after the addition of 1% and 1.5 % BaTiO3 NPs (Figures 3-5).

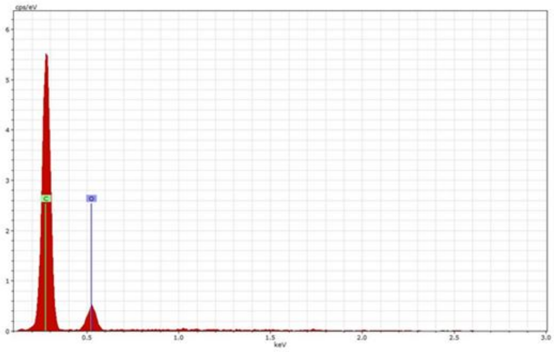

Figure 3: EDS diagram for soft liner without addition.

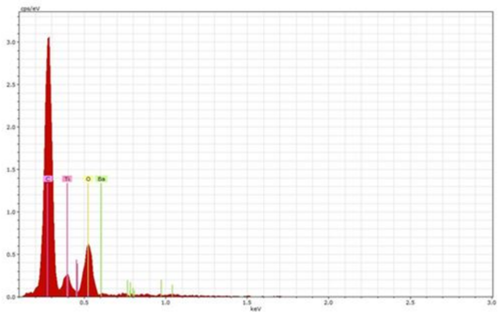

Figure 4: EDS diagram for soft liner after addition of 1% by wt. $BaTiO_3$ NPs.

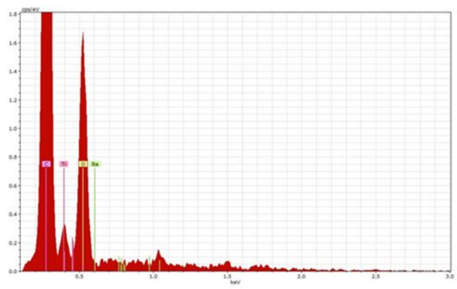

Figure 5: EDS diagram for soft liner after addition of 1.5% by wt. BaTiO3 NPs.

Candida albicans **adherence test results: under an inverted light microscope stained specimens for each group were inspected, the control group has a higher mean value (41.49), while the experimental group (1.5% by wt. of $BaTiO_3$ NPs) has the lowest mean value (6.65) (Figure 6).**

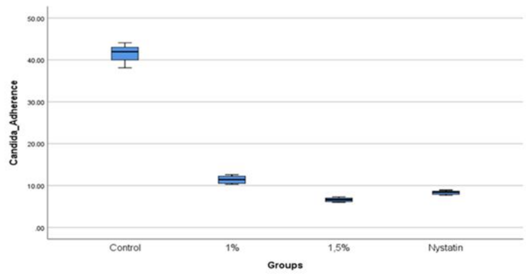

Figure 6: box-plot chart for ***Candida albicans*** **adherence test.**

Comparison of means of ***candida albicans*** **adherence test results using one way ANOVA table test showed a highly significant decrease in** ***Candida albicans*** **adherence to the surface of soft denture liner as shown in table 1.**

Table 1: Mean of Candida albicans adherence test and one-way ANOVA table for all studied groups.

Group	N	Mean	SD	SE	Minimum	Maximum	F
Control	10	41.492	1.89672	0.59979	38.1	44.1	2293.417
1% $BaTiO_3$ nps	10	11.418	0.85008	0.26882	10.3	12.6	
1.5% $BaTiO_3$ nps	10	6.6567	0.4622	0.14616	6	7.3	
Nystatin	10	8.325	0.44074	0.13937	7.68	8.91	

According to the results of Levene's test for data homogeneity, which show highly significant differences between groups, the Games-Howell post-hoc test was chosen for ***candida*** **adherence multiple comparisons (Table 2).**

Table 2: Games-Howell post hoc test for *Candida albicans* adherence.

Tested groups		Mean difference	Sig
Control	1%	30.07*	0.000HS
	1.50%	34.83*	0.000HS
	Nystatin	33.16*	0.000HS
1%	1.50%	4.76*	0.000HS
	Nystatin	3.09*	0.000HS
1.50%	Nystatin	-1.66*	0.000HS

*The mean difference is significant at the 0.05 level.

Radiopacity test results: The control group had the highest mean score of optical density (1.3112), while the experimental group had the lowest mean score (1.5% incorporation of BaTiO3 NP) (1.0353) (Figure 7).

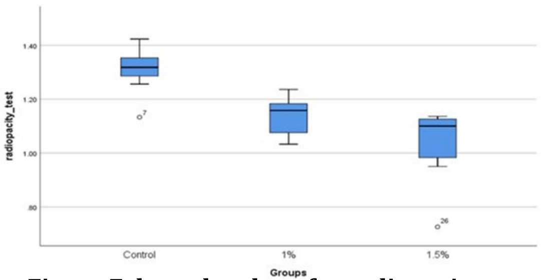

Figure 7: box-plot chart for radiopacity test results.

A comparison of the means of all groups' radiopacity test results using one-way ANOVA table test showed a highly significant increase in radiopacity in experimental groups when compared to the control group as shown in table 3.

Table 3: Mean for radiopacity test and one-way ANOVA table for all tested groups.

Groups	N	Mean	SD	SE	Minimum	Maximum	F	P value
Control	10	1.3112	0.08158	0.0258	1.13	1.42	20.736	0.000HS
1% BaTiO3 nps	10	1.1377	0.0688	0.02176	1.03	1.24		
1.5% BaTiO3 nps	10	1.0353	0.12943	0.04093	0.73	1.14		

Bonferroni post-hoc test was used to compare mean value between each two groups (Table 4), there was high significant difference between (control and 1% groups) and (control and 1.5% groups), while there was non-significant difference between 1% and 1.5% groups.

Table 4: Bonferroni post hoc test for radiopacity test.

Tested groups		Mean difference	Sig.
Control	1%	.17350*	0.001 HS
	1.50%	.27590*	0.000 HS
1%	1.50%	0.1024	0.077 NS

Surface hardness test results: Highest mean value of surface hardness test was for the 1.5% incorporation of $BaTiO_3$ NP group (50.1600) while the lowest mean value was for control group (48.5200) as shown in Figure 8.

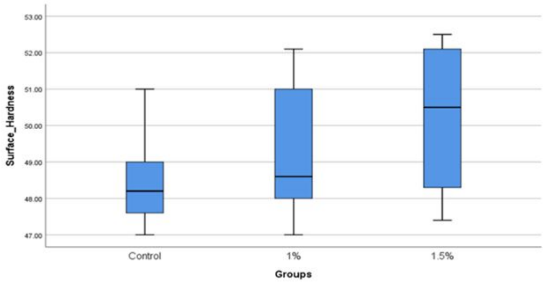

Figure 8: box-plot chart for Shor A surface hardness test results.

Comparison of means of surface hardness test results of all groups using one way ANOVA table test revealed a non-significant increase in surface hardness of soft lining material after addition of 1% and 1.5% by wt. barium titanate nano particles as shown in table 5.

Group	N	Mean	SD	SE	Minimum	Maximum	F	P value
Control	10	48.52	1.30792	0.4136	47	51	2.283	0.121NS
1% BaTiO3 nps	10	49.28	1.90193	0.60144	47	52.1		
1.5% BaTiO3 nps	10	50.16	1.87688	0.59352	47.4	52.5		

Table 5: Mean for surface hardness test and one-way ANOVA table for all tested groups.

DISCUSSION

Liners are commonly used in dentistry to alter prosthetic surfaces that come into contact with soft tissues in the oral cavity. Soft liners are a type of flexible material that is used to reline denture base surfaces that come into contact with the occlusal stress bearing oral mucosa [31].

Several studies have tried to combat fungi colonization by incorporating antifungal agents into the soft denture liner material, for example; when compared to conventional organic antimicrobial agents, metal oxide nanoparticles such as TiO_2, MgO, and ZnO are thought to be superior antimicrobial agents in terms of safety, durability, and heat resistance [32,33].

The current study tried to enhance soft lining material properties against *C. albicans* adherence by incorporating $BaTiO_3$ NPs into soft liner.

In *Candida albicans* adherence test incorporation of $BaTiO_3$ NP powder in two different precents of weight (1%, 1.5%) caused a highly significant decrease in *Candida albicans* adherence to acrylic based heat cured soft liner compared to control group and in comparison, with Nystatin control positive group the 1.5% group showed significant difference in decreasing *Candida albicans* adherence.

$BaTiO_3$'s antifungal mode of action include two proposed method: Due to the increase in positive surface potential, which may have resulted in bacteria being neutralized on their surfaces, resulting in the creation of ROS (Reactive Oxygen Species), that can cause membrane and DNA damage [34].

When *Candida albicans* is exposed to nano $BaTiO_3$, the nanoparticles can covalently connect to the membrane components. This results in a disturbance of the cell wall architecture, which ultimately results in the death of *Candida albicans via* suppression of ergosterol production.

In radiopacity test a highly significant increase in radiopacity observed after addition of barium titanate nanoparticles to soft denture liner compared to control group. This finding can be explained that barium and titanium have high atomic number (56 for barium and 22 for titanium) compared to the elements of polymer which produce more radiopacity when added to the polymer.

Furthermore, the addition of barium titanate nanoparticles to polymer should result in an increase in density, as NBT has a higher density than polymer. (6.08 g/cm^3, 0.906 g/cm^3 respectively) [35], this increase in material density result in increased radiopacity of the material as the radiopacity determined by the size, density, chemical composition of the filler molecules, and their concentration within the polymer matrix [36].

In shore a surface hardness test the results revealed that there is no significant increase in surface hardness between control group and experimental groups, thus they were within the ISO specification limit for long-term soft lining materials. Because only trace amounts of $BaTiO_3$ NPs were introduced to the soft denture liner, they had no discernible effect on the material's surface hardness due to low network density and small particle size of $BaTiO_3$ NPs.

CONCLUSION

The results of this study indicate that integration of $BaTiO_3$ nanoparticles into soft denture liner resulted in a significantly substantial increase in antifungal activity as it reduced the adherence of *Candida albicans* to soft denture liner. Also, a highly significant increase in radiopacity of denture liner resulted from $BaTiO_3$ NPs incorporation, while there was non-significant increase in surface hardness.

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