RESEARCH PAPER

The Effect of Boron Nitride Nanoparticles Incorporation in to Heat Cured Soft Denture Lining Material on *Candida Albicans* Adherence and Other Properties

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ABSTRACT

Long-term acrylic soft denture lining materials have many advantages in prosthetic dentistry, but the nature of these materials makes them subject to different problems. One of these widely spread problems was candida albicans. Because of the difficulty in killing fungal cells and the deep penetration of fungi inside the soft denture lining material, researchers tried to find other ways to treat this problem. They depended on nanoparticles to improve the antifungal and mechanical properties of the soft denture lining material. In this study Boron nitride nano particles were added to acrylic soft liner in attempt to decrease *Candida albicans* adherence and evaluate its effect on other properties which are: Surface roughness and surface hardness. According to the results of the pilot study, we chose 1.5% and 2% by weight of BN NPs as the most suitable percentages added to the soft lining material.Main: 90 specimens were prepared, 30 specimens for each test, which are the candida albicance adherence test, roughness test, and hardness test. Boron Nitride Nanoparticles were spread through monomer by sonication using a sonicator probe, then the soft liner powder was added to the sonicated monomer with nanoparticles, and the specimens were taken to investigate Boron Nitride Nanoparticles efficiency as antifungal activity and as a mechanical property improver. When boron nitride nanoparticles were added to the soft lining material, the adhesion of Candida albicans was high significantly reduced, without significantly increasing the surface roughness. According to the results of the hardness test, the inclusion of BN NPs was high significantly increased the soft liner's hardness. The antifungal activities of soft lining materials were greatly enhanced due to BN NPs powder's potent antifungal action against C. albicans. There was no significant increase in surface roughness, and hardness was highly increased after incorporation of BN NPs into heat-cured soft denture lining material.

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INTRODUCTION

A large majority of patients (93%) felt more at ease wearing a soft-lined denture, according to lengthy retrospective research. The same survey also found that a large majority of patients (95%) would choose to have their new dentures softly relined [1]. The term "soft liners" refers to a group of resilient dental materials that are applied on the fitting surfaces of the denture to form a soft padded layer between the hard denture base

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and the contacting occlusal surfaces of the oral mucosa . [2]

O'Brien categorized soft liners as either silicone- or acrylic-based depending on their material composition. [3] denture-relining materials have relatively poor antimicrobial properties, which can cause denture stomatitis, a common oral mucosal disease [4].

Despite the advantages of soft liners, they are more difficult to clean than hard denture bases because they cannot be cleaned by using mechanical brushing [5,6]. In addition to having reduced resilience and water sorption,[5,6] they are easily degradable, prone to the accumulation of microorganisms, and can contribute to the progression of pathological processes that can limit the treatment options for existing infections [7]. Soft liners that lack antifungal properties allow the formation of biofilm, which is difficult to remove even using denture cleansers [8]. Recently, to overcome this problem, soft liners with antifungal properties have been formulated and proven to reduce *C. albicans* adhesion and prevent Denture- induced stomatitis [9].

A major health concern of the 21st century is the rise of multi-drug resistant pathogenic microbial species. Recent technological advancements have led to considerable opportunities for low-dimensional materials (LDMs) as potential next-generation antimicrobials. LDMs have demonstrated antimicrobial behaviour towards a variety of pathogenic bacterial and fungal cells, due to their unique physicochemical properties [15]. Adding BaTiO3 nanoparticles to soft denture liners decreased Candida albicans' ability to stick to the liners and dramatically increased the liners' anti fungal action [10].

Chitosan micro-particles (1.5%, 2.5% and 3.5% by wt.) addition in to room temperature vulcanized silicone elastomer material was revealed a high significant decrease in the numbers of *C.albicans* cells adhered to experimental group specimens, and the disk diffusion test showed high significance increase in the inhibition zone measurement as the chitosan concentrations increase [11].

Chemical modifications of one-dimensional (1D) materials can enhance the antimicrobial activity to materials with little to no inherent efficiency, polymer functionalism Boron nitride nanoparticles were shown to have an increased efficacy compared to pristine Boron nitride nanoparticles [12].

The antibacterial activity of hBNs was reported on Candida sp. M25, S. mutans 3.3, standard S. mutans ATCC 25175, S. pasteuri M3, E. coli DH5, and E. coli ATCC 25922 [13,14]. One of the nondestructive tests for determining whether a resilient material is suitable for use as a liner is the hardness test (shore A), which measures the response force of the tested material to indentation [16]. Rougher surfaces are more conducive to biofilm formation because they increase microbial adherence [17].

h-BN with a layered structure analogous to graphite has been widely studied due to its features such as an ultra-flat surface and a highly stable structure as well as remarkable thermal conductivity and insulating properties [18,19]. The addition of filler (Al2O3 powder) in to heat cured PMMA denture base material increase in surface hardness while the surface roughness was not affected except a significant increase was observed in surface roughness by the addition of 10% Al2O3 to PMMA [20]. The addition of nanofillers (mixtureZrO_2-Al_2O_3) at 2wt.% to PMMA led to increase of surface hardness beyond that of pure PMMA, statistically was not-significant, while there is a significant increase in surface roughness [21].

In this study we aimed to evaluate anti fungal activity of BN NPs incorporated in to acrylic based soft denture lining material, in addition to its effect on surface roughness and hardness of soft lining material.

MATERIALS AND METHODS

In this investigation, we used a heat-cured soft denture liner made of polyethylmethacrylate (called "Moon star"). The results of the adhesion test with Candida albicans suggested that BN NPs at concentrations between 1.5% by wt. and 2% by wt. would have the greatest impact.

Sampling

Ninety samples were prepared, and three sets were created according to the intended analysis. Next, we subdivided each group into three categories based on the amount of BN NPs they contained (control = 0%, 1.5% by wt. BN NPs, and 2% by wt. BN NPs).

Acrylic soft liner preparation

Following the manufacturer's instructions, soft liner is blended in a dry glass container with a powder liquid ratio of (10g/ 7.8ml) until at the dough stage is reached, Then, the mixture is put into a stone mold and squeezed under 100 kgf/ cm2 in a hydraulic press to distribute the soft lining evenly.Following the manufacturer's directions, we heated a flask containing a sample of the soft liner to 100 ˚C and held it at that temperature for 20 minutes.

After taking samples from their flasks, we cut off extra material using a sharp blade, polished them with a fine-grit polishing burs and sandpaper, and stored them in distilled water in an incubator at 37 degrees Celsius for a whole two days.

Utilization of Nano-Sized Fillers

The BN NPs used in the various experiments were weighed in a dry and sanitized glass jar utilizing a digital balance with 0.001 meticulousness before Soft liner's solvent monomer was included into the mix. It was mixed using a sonication probe at 240 W, 24 KHz [KQJS-300 China] for 3 minutes with BN NPs to breakdown it into nanoparticles. In accordance with manufacturer's instructions, Powder was added, blended, loaded, and cured thereafter.

To examine whether or not the PEMA soft liner and the BN NPs have any kind of chemical interaction, an FTIR spectrum was taken. .Nanoparticle dispersion degree within the PEMA the heat cured soft liner matrix was also evaluated using field emission scanning electron microscopy (FE-SEM) on samples from the control as well as the groups experimented (1.5% by wt. and 2% by wt. BN NPs). The percentages of the composite fillers in terms of weight and atoms were also determined by Energy Dispersive X-ray spectroscopy [EDS].

Candida albicans adherence test

For the Candida albicans adhesion test, thirty disc-shaped samples of soft liner were cut to 10mm in diameter and 2mm in thickness [22].

Isolation and identification of *Candida albicans*

Using a sterile cotton swab,Candida albicans from patients was isolated who have "denture-induced stomatitis" complaints . The specimen was then promptly inoculated on Sabouraud Dextrose Agar (SDA) and incubated at 37oC for 48 hours [23].

Candida albicans was identified using morphological analysis; it has a pearl-shaped, smooth, creamy, and pasty look [24]. Gram staining was used for microscopic analysis, which was performed using a light microscope (Olympus, Japan). Then, a The Fully Automated VITEK 2 System using technology of fluorescence-based for microbe verification was used to conduct biochemical analysis [25]..

Candida albicans adherence test procedure

The manufacturer's instructions for preparing Sabouraud dextrose broth were followed, and a small amount of *candida albicans* was included in the medium in order to make 0.5 McFarland suspension [26]. Soft lining samples that had been autoclaved were inserted in sterile tubes with a broth prepared as well as allowed to incubate at the room temperature for 1 hour. Samples were then removed from their tubes then washed in phosphate buffered saline for 1 minute.Gently shaking to remove non-adherent yeast cells and drying with filter paper. Cells of Candida albicans that had adhered to the soft liner surface specimen in methanol were fixed, for 1 minute stained with crystal violet, washed in solution PBS, allowed on filter paper to dry, then examined under light inverted microscope [27,28]. The soft liner samples were examined using an inverted light microscope, with three fields obtained for each sample. Candida albicans cells were counted in each field, and the mean of the three counts was used to characterize the sample.

Surface roughness test

Surface roughness test specimens of soft liner were manufactured with dimensions of 65mm in length, 10mm in width, and 2.5mm in thickness. The profilometer was used to measure the roughness of the surface in accordance with ANSI/ ADA standard No. 12, 1999.

The thirty specimens of surface roughness were measured using a profilometer device (Time3200/ TR200, China), This instrument consists of a diamond needle (stylus) with a pointed, sensitive tip that is used to trace the profile of surface irregularities. Each sample was measured three times, and the average was determined.

Surface hardness test

Soft denture lining specimens were prepared for the Shore A hardness test using disk-shaped specimens that were 6mm thick and 35mm in diameter, as specified by ISO-10139-2 (2016). The thirty specimens were used to measure a

Shore A durometer [Time group-TH200, China] [29].Every sample was read five times, Following each penetration, there was a 5-second period of contact, and the average was used as the value of test.

Analytical statistics

Computer software [version 21] was used for analysis the study´s results, SPSS [social science Statistical package], in addition to inferential statistics and descriptive. A one-way [variance analysis] ANOVA test was used, in addition to inferential statistics and descriptive.(variance analysis) test was used ANOVA one-way. A statistical "P" value of > 0.05 was intended to be non-significant statistically.It was considered significant when the "P" value was 0.05, and a highly significant 0.01 "P" value.

RESULTS AND DISCUSSION

FTIR analysis: The FTIR spectra clearly reveal that the BN NPs and acrylic soft liner have not reacted chemically (Fig. 1]).

Field emission electron Scanning microscope (FE-SEM)

Comparison of soft liner microstructure before and after dosing with 1.5% and 2% by weight Boron Nitride Nanoparticles powder, as shown by field emission scanning electron microscopy (FE-SEM),The findings demonstrate the uniform dispersion of nanoparticles throughout the matrix

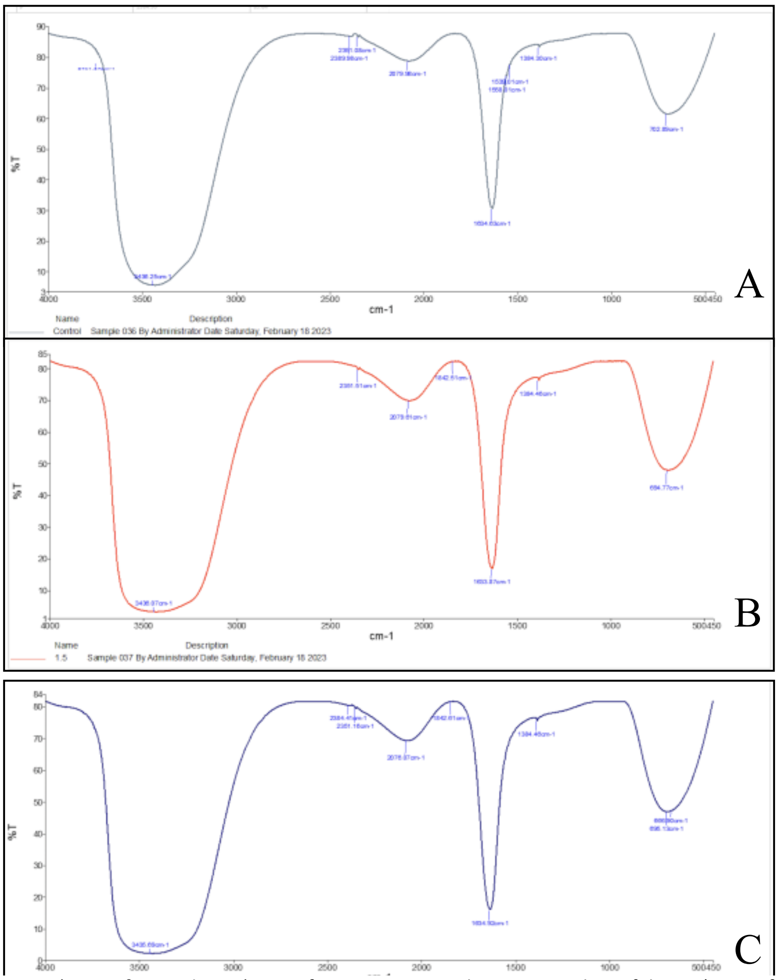

Fig. 1. A) FTIR of control 0% B) FTIR of 1.5% Boron Nitride Nanoparticles soft liner C) FTIR of 2% Boron Nitride Nanoparticles soft liner .

of the lining material, with just a little degree of filler aggregation (Fig. 2).

Energy - dispersive x-ray spectroscopy (EDS)

A device gives elemental analysis of the specimen before and after the addition of BN NPs, in control group before addition of BN NPs the element present Carbon, Oxygen, Silicon. After addition of BN NPs it appears Boron, Nitrogen in addition to the presence of substantial elements of the original soft liner material with increase in amount of Boron and Nitrogen with 2% by wt. BN NPs incorporation (Fig. 3).

Candida albicans adherence test results

Stained samples were examined for each group

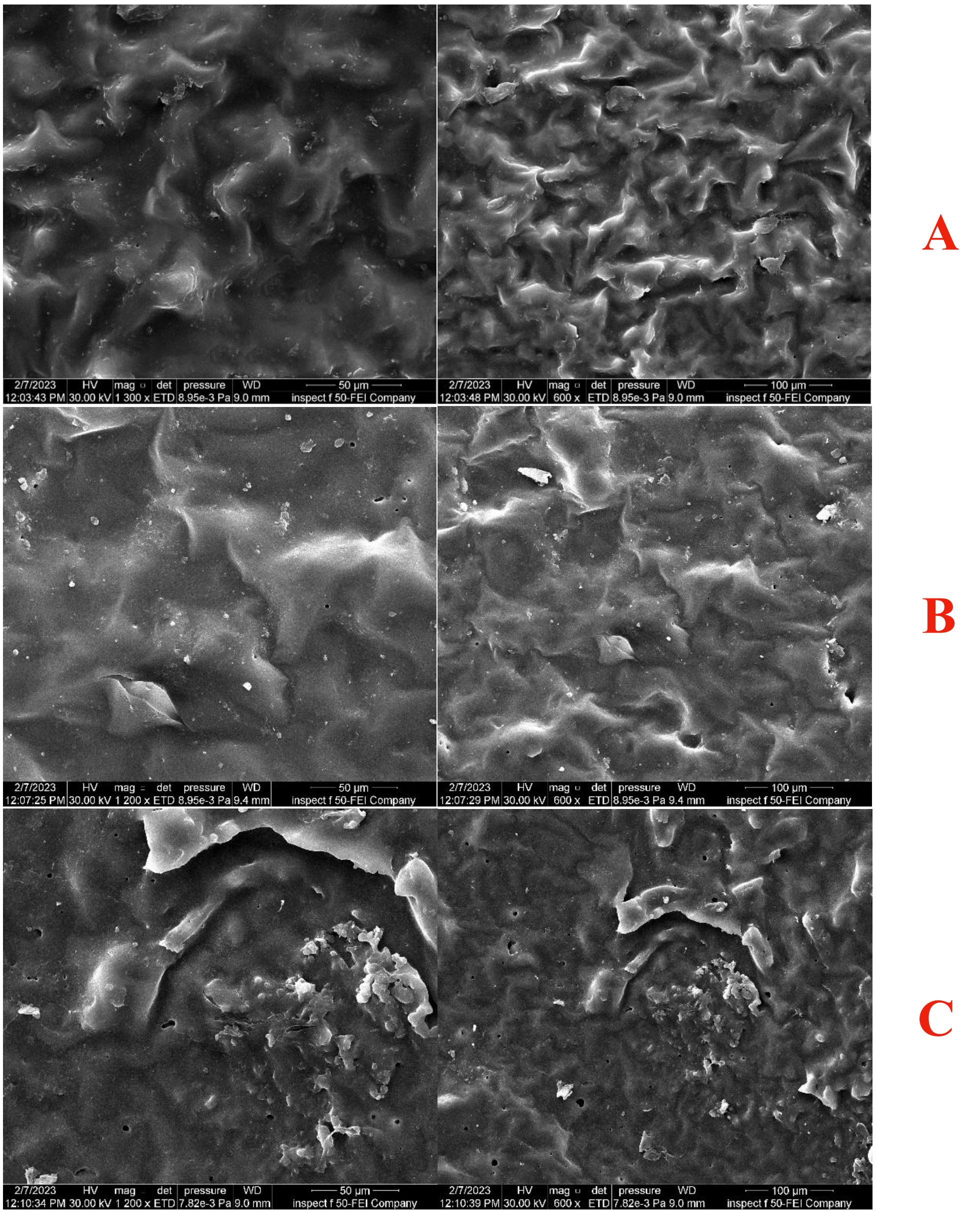

Fig. 2. A) Control specimens B) FE-SEM of 1.5% of Boron Nitride Nanoparticles soft liner image C) FE-SEM of 2% of Boron Nitride Nanoparticles soft liner image.

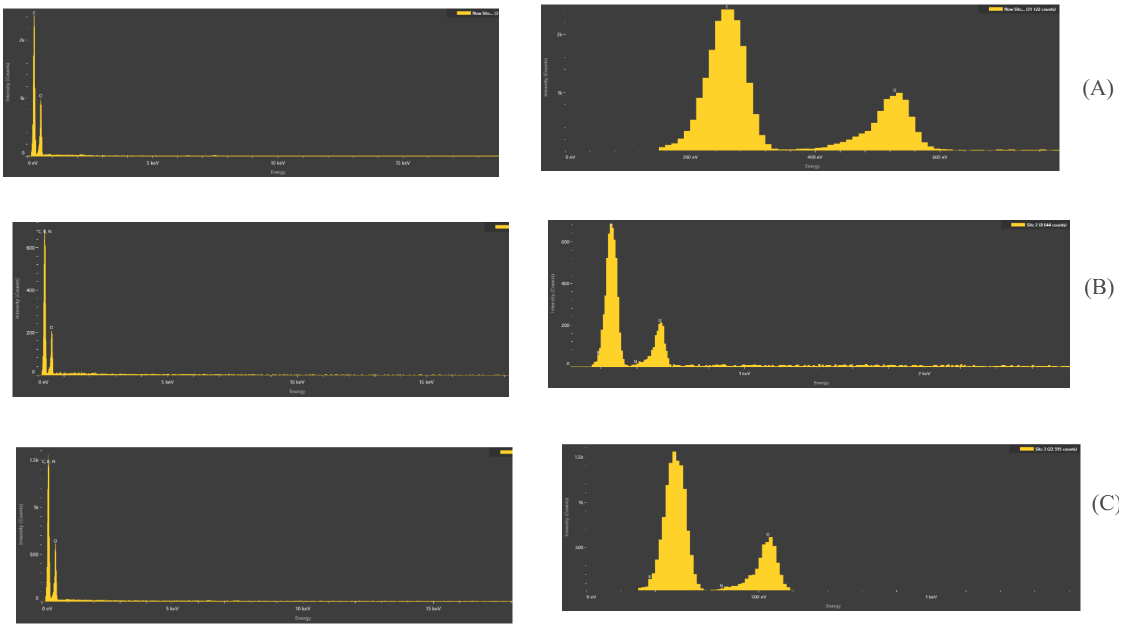

Fig. 3. EDS diagram for soft liner A) before the addition of BN NPs; B) After addition of 1.5% by wt. BN NPs; C) After addition of 2% by wt. BN NPs.

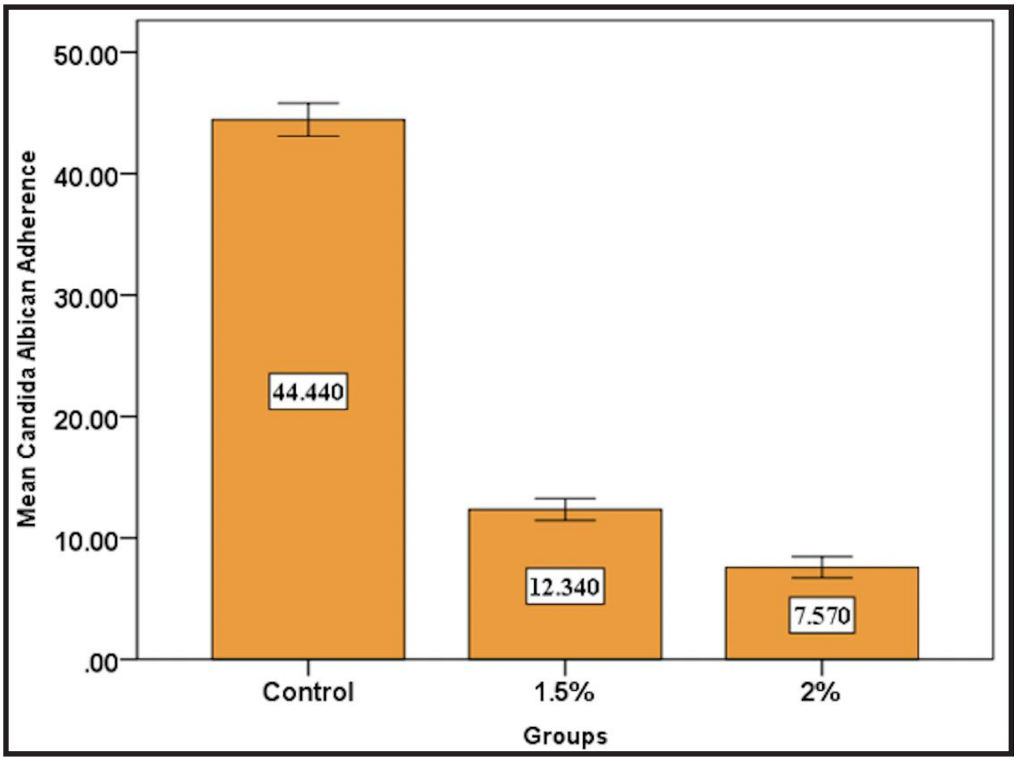

Fig. 4. Bar chart for Candida albicans adherence test.

using an inverted light microscope. A higher mean value was found in the control group, The BN NP's incorporation of 1.5% by weight comes after the control group in its mean value, while the incorporation of soft liner with 2% BN NPs introduced the lowest mean value . As shown in Fig. 4.

Table 1 shows the findings of a one-way analysis of variance (ANOVA) on the means of test results for Candida albicans adhesion to the soft denture liner surface.

The post hoc Tukey Honestly Significant Difference (HSD) test was performed to compare the means of the groups under study, and the results showed that there was a statistically high significant difference between the groups. As shown in Table 2.

Hardness test results

The findings of Shore A hardness test showed that specimens containing 2% BN NPs had the greatest mean value, followed by specimens containing 1.5% BN NPs, then the control group. These results are shown in Fig. 5.

Test of significance for Shore A using one-way (ANOVA) hardness test was conducted, and the findings revealed that between all of the groups tested, there was a high significant difference, as indicated in Table 3.

The post hoc Tukey Honestly Significant Difference (HSD) test was performed to compare the means of the groups under study, and the results showed that there was a statistically high significant difference between the groups. As shown in Table 4.

Surface roughness test

As can be seen in Fig. 6, the roughness test results showed that specimens containing 2% BN NPs had the value with the highest mean, then specimens with 1.5% BN NPs, Finally the control group. The results for all groups are quite close.

There was no statistically significant difference in surface roughness between any of the groups evaluated in a one-way analysis of variance (ANOVA), as demonstrated in Table 5.

Table 1. For all studied groups mean of the *Candida albicans* adherence test and ANOVA table

Groups	N	Mean	±SD	±SE	Minimum	Maximum	F	P Value
Control	10	44.440	1.896	0.599	42.000	48.000		
1.5% BN NPs	10	12.340	1.260	0.398	10.700	14.600	1817.629	0.000 sig.
2% BN NPs	10	7.570	1.207	0.382	6.000	10.000		

Table 2. Multiple comparisons of *Candida albicans* adherence test using Tukey HSD between percentages.

(I) Groups	(J) Groups	difference Mean (I-J)	P value
Control	1.5% BN NPs	32.10000	0.000 Sig.
	2% BN NPs	36.87000	0.000 Sig.
1.5 % BN NPs	2% BN NPs	4.77000	0.000 Sig.

Widespread usage of liners to modify the contour of dental prosthetics that will come into touch with the mouth's delicate tissues. [30]. Breakdown in adhesion, rough surfaces,

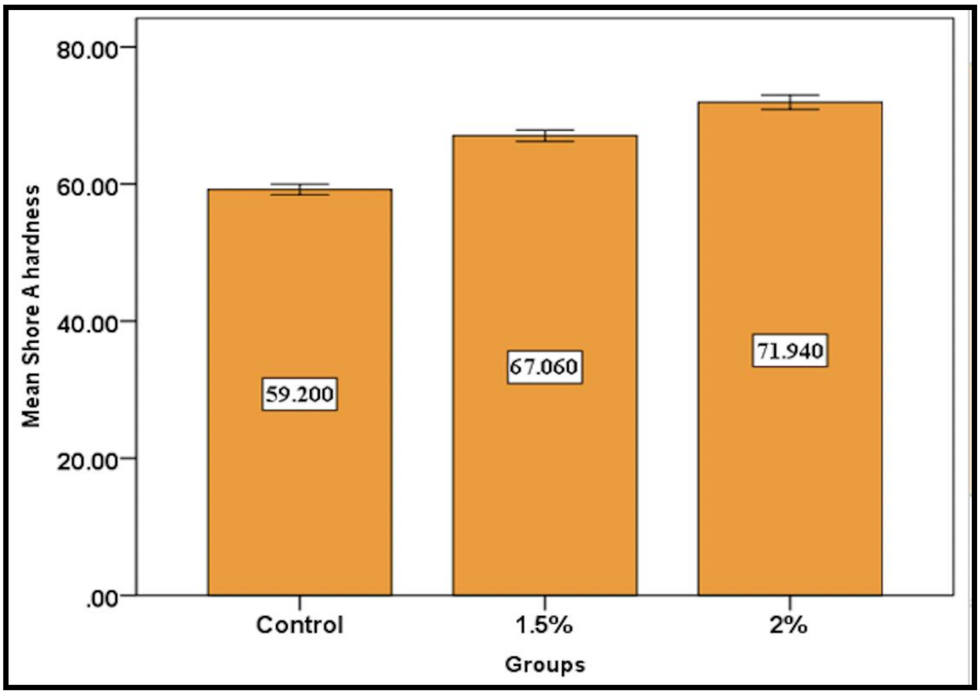

Fig. 5. Bar chart represents the mean value of hardness test for each group.

Table 3. For all tested groups ANOVA and Shore a hardness test means.

Groups	N	Mean	±SD	±SE	Minimum	Maximum	F	P value
Control	10	59.200	1.071	0.339	57.600	60.800		
1.5% BN NPs	10	67.060	1.170	0.370	65.000	68.600	262.558	0.000 Sig.
2% BN NPs	10	71.940	1.485	0.470	70.000	74.000		

Table 4. Multiple comparisons of Shore A hardness test between percentages using Tukey HSD.

(I) Groups	(J) Groups	difference Mean (I-J)	P value
Control	1.5% BN NPs	-7.860	0.000 Sig.
	2% BN NPs	-12.740	0.000 Sig.
1.5% BN NPs	2% BN NPs	-4.880	0.000 Sig.

and variations in hardness are all conducive to bacteria collection, which in turn reduces the liner's longevity and may lead to oral health problems including denture stomatitis. [31] Low-dimensional materials (LDMs) have attracted a lot of attention lately as promising anti-microbial substances [32, 33].

In the current study, we tried to improve the properties of soft lining material including anti fungal, surface hardness and surface roughness by addition of BN NPs in different percentages.

Our study clarified that *candida albicans* adherence to the soft denture lining material was decreased, which was highly significant after addition BN NPs of 1.5% by wt. and 2% by wt. to the soft denture lining material

Boric acid is not intrinsically as potent as many antifungal pharmaceuticals, but may nevertheless derive clinical effect from high doses and the continued release from incompletely dissolved drugs, which are replenished daily for 14 days in typical use.Boric acid is fungistatic to fungicidal

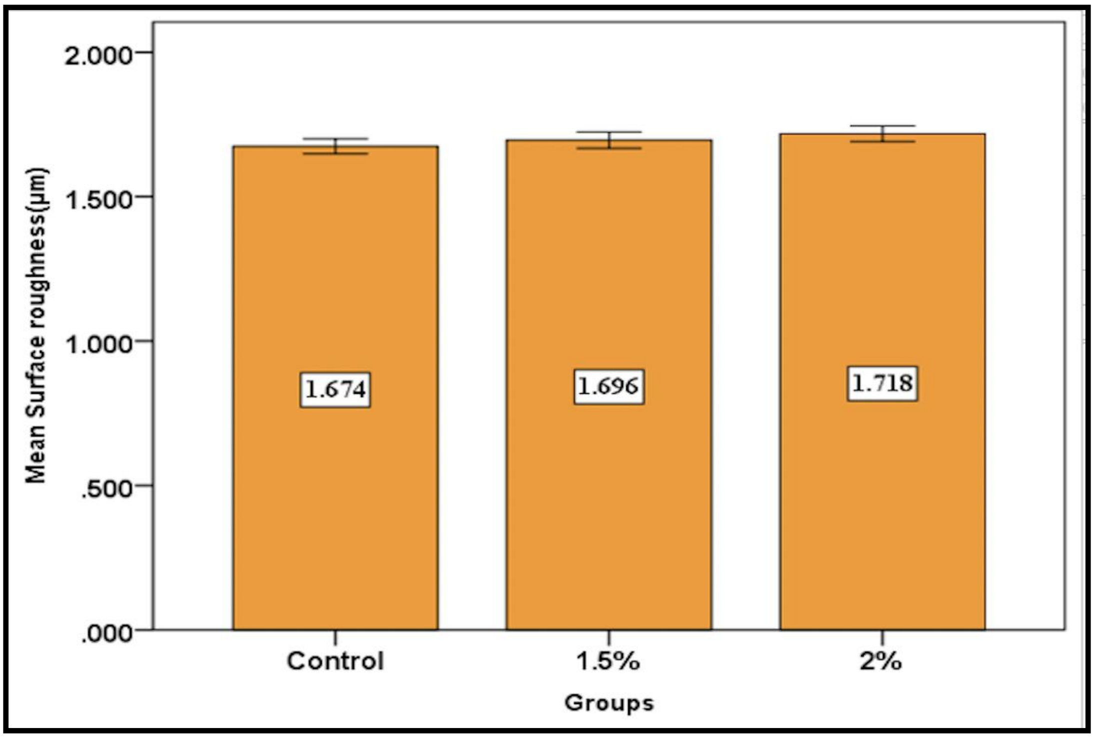

Fig. 6. Bar chart represents the mean value of roughness test for each group.

Table 5. For all tested groups ANOVA and means of surface roughness test.

Groups	N	Mean	±SD	±SE	Minimum	Maximum	F	P value
Control	10	1.674	0.037	0.012	1.623	1.713		
1.5% BN NPs	10	1.696	0.039	0.012	1.640	1.750	3.283	0.053 NS
2% BN NPs	10	1.718	0.038	0.012	1.660	1.770		

depending on concentration and temperature. Inhibition of oxidative metabolism appears to be a key antifungal mechanism, but inhibition of virulence probably contributes to therapeutic efficacy in vivo.Depending on temperature and concentration, boric acid is either fungistatic or fungicidal. Although suppression of virulence likely adds to treatment efficiency in vivo, inhibition of oxidative metabolism seems to be a crucial antifungal mechanism [34].

Boron Nitride has bacteria and fungi suppression mechanisms one of them chemical ROS formation [35]. Reactive oxygen species (ROS) can cause damage to important cellular components including proteins, nucleic acids and phospholipids, resulting in cell lysis [36].

It has been found that the presence of a boron atom is key to an antifungal agent being developed to treat infections of fingernails and toenails. The antifungal compound works by clogging up an enzyme involved in translating the fungal DNA into its protein products, thereby preventing protein synthesis [37]. Boric acid inhibition has been rescued by ribose, NAD and tryptophan providing information on the The mechanistic pathway behind boron [38]. The topical application of boron alone or in combination with other anti-fungals could be an alternative treatment for superficial mycosis in patients who respond to standard anti-fungals [39]. Results of the study showed that high significant increase in hardness with addition of Boron Nitride Nanoparticles compared with control group. The increase in hardness is directly proportional with increase in Boron Nitride Nanoparticles concentration. The elastic property result from inter-molecular force or inter-atomic of the material that are responsible for the property of elasticity. The stronger attraction forces, the more values of the elastic modulus and the more rigid or stiffness material [40].

This result agree with previous study by Abdulmajeed and Abdulbaqi in 2021 who stated increase in surface hardness of acrylic soft denture liner after the addition of calcium carbonate nanoparticles [41]. Our result also agree with previous study by Abdulbaqi et al in 2022 who stated the increase in surface hardness of acrylic soft denture liner after the addition of Yttrium oxide nanoparticles [42].

The nanoparticle dispersion inside the matrix may be responsible for the increase in hardness. The particles cluster together in the gaps of the soft-lining matrix as the interparticle distance decreases and the bonding strength between the particles increases, which in turn causes an increase in hardness [43-45].

The results of this study showed that surface roughness of soft denture liner which was not significantly increased after the addition of BN NPs. When small percentages of Boron Nitride nanoparticles were added to soft denture liner only few particles will be included with the surface of the specimen which didn't influence in surface roughness as the surface roughness test is concerned with outer surface and not with the inner surface of composite, Since BN NPs have very small sized particles and well dispersion, and this agreed with the result of Abdulmajeed and Abdulbaqi, 2021 study who found that there is slight increase in acrylic soft liner surface roughness after the addition of $CaCO_3$ NPs but this increase statistically non-significant [41].

CONCLUSION

The result of this study shows highly significant increase in anti fungal activity of BN NPs after its addition to the soft lining material, with high significant increase in hardness of soft denture lining material, with non significant increase in surface roughness.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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