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Surface Hardness and Fourier Transform Infrared Spectroscopical Characterization of Bioactive Modified Chairside Hard Denture Reliner

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Article information

Abstract

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Aims: The study seeks to investigate the hardness and chemical structure of the MWCNTs-Nanonystatin-modified hard chairside denture reliner after nanosizing nystatin macromolecules to be conjugated with MWCNTs for loading into chairside hard denture reliners. Materials and Methods: The examined material was chairside hard denture reliner (CHDR) (Rebase II). Tokuyama[®]. Japan. The nano additives applied were Nystatin (Nys). SDI (Samirraa' Drugs, Iraq) and Multiwalled carbon nanotubes (MWCNT, 95%, VCN materials). Chairside relining material was manufactured following the manufacturers' specifications. Surface hardness was tested using a shore D durometer tester, while FTIR Spectroscopy was conducted using an IRAFFINITY-1S-FTIR spectrophotometer (SHIMADZU, Japan). The chairside hard denture reliner specimens were divided into (a control group,5 specimens) and a modified chairside hard denture reliner (MWCNTs-Nys-CHDR at 0.025%, 0.05%, and 0.1% by weight, 5 specimens for each group). The CHDR specimens have dimensions of 10×10×3.3 ±0.2mm. Total number of samples in this study was 40 samples. Results: The bioactive modification of CHDR resulted in increased hardness without changing the chemical structure of the materials in comparison to the control group. Conclusions: The bioactive modification of CHDR led to improved hardness, without any chemical modifications.

الخصائص الطيفية والصلابه لماده تبطين طقم الاسنان الصلب بعد التعديل

الحيوي

الملخص

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INTRODUCTION

Denture-bearing regions undergo significant changes after tooth extraction, necessitating the relining of removable prostheses to provide the correct fit and support. Auto polymerizing reline resins allow dentists to quickly reline removable prostheses inside the mouth, saving time and accurately replicating the denture base with oral soft tissue characteristics (1). However, the earliest relining products were exclusively composed of the monomer methyl methacrylate, which exhibited increased porosity, cytotoxicity, heat emission, decreased mechanical strength, and colour longevity compared to heat polymerized denture base acrylic resins ^(2 & 3). To address these constraints, alternative polymers such as polyethyl methacrylate (PEMA) and monofunctional monomers such as butyl methacrylate and isobutyl methacrylate (IBMA) have been used to address these constraints. However, these chairside relining materials still exhibit lower physicochemical and mechanical properties compared to heat-polymerized denture base resins (3 & 4).

Researchers are continuously exploring various methods to find the optimal combination of chemical, physicomechanical, and cosmetic properties for denture base materials and hard denture reliners. The challenge lies in enhancing specific characteristics while maintaining the integrity of other characteristics (2, 5-7). Surface hardness refers to the material's ability to withstand scratching or abrasion, which can lead to increased surface roughness and the adhesion of microorganisms ⁽⁸⁾. Nanotechnology has become an essential tool in biomedicine, particularly in medication delivery, as seen in the use of nanotubes and nanoparticles, Multi-Walled Carbon Nanotubes (MWCNts) mixed with PMMA to create a nano additive that can effectively kill microbes without the need for drugs ⁽⁹⁾. Nanotubes' weak adhesion to the resin matrix and tendency to clump together resulting in inadequate dispersion within the resin. Sonication and surfactant improve the spreading of carbon nanotubes (10). New efforts have been made to load pharmaceuticals onto Multi-Walled Carbon Nanotubes (MWCNts) for controlled and prolonged drug release. Nystatin, a popular topical treatment for fungal infections, may decrease patient compliance due to its high administration frequency (11). This study aims create nystatin-loaded Multi-Walled to Carbon Nanotubes (MWCNts) reliner to improve its hardness chemical and composition. The null hypothesis posited that the inclusion of multi-walled carbon

nanotubes impregnated with Nystatin nanoparticles at 0.025%, 0.05%, and 0.1% concentrations by weight into the Chairside Hard denture reliner (Rebase II) would have no impact on the surface hardness and chemical composition of the bioactivity modified denture reliner.

MATERIALS AND METHODS

The research ethics committee of the College of Dentistry at University of Mosul in Iraq (UoM. Dent. 23/13) granted approval for this study on 9/4/2023.

The nystatin macromolecules were reduced to nano size and then conjugated with MWCNTs after it is activated and functionalized to be loaded into chairside hard denture reliner ⁽⁹⁻¹¹⁾. This involved a dual drug loading process that had been applied at two levels: initially, the nanoparticle drug conjugates (as nano nystatin loaded within MWCNTs), and subsequently, the polymer-drug conjugates (as MWCNTs-Nys loaded within modified chairside hard denture reliner).

Reliner sample preparation involved creating specimens by mixing powder and liquid, using a specially designed metal mold was used as the test's dimensions (Figure 1), and applying light pressure to remove excess material (4 kg as prescribed by ADA no 17., 2006) ⁽¹²⁾. The specimens were left undisturbed until polymerization was complete and then refined using 400-grit silicon carbide paper. They were then immersed in sterile distilled water and kept at 37°C for 48 hours for conditioning. The process adhered to manufacturer instructions.



Figure 1: Metal Moldes.

1. Surface hardness:

A total of 20 chairside hard denture reliner samples have been utilized. This included a control group with 5 specimens, and three experimental groups using MWCNTs-Nys-CHDR at concentrations of 0.025%, 0.05%, and 0.1% by weight, each group consisting of 5 specimens. The specimens will be constructed with dimensions of $10 \times 10 \times 3.3 \pm 0.2$ mm (length, width, and height accordingly). Five measurements on various regions of each specimen will determine the average value ⁽¹²⁾.

The surface hardness of the specimens was measured using a Durometer hardness tester of type D (Shaw, China). A spherical steel ball is utilized as an indenter with a diameter of 1.25mm. The specimen will be positioned on the flat surface of the device, while the needle will be maintained at a distance of 12 mm from the specimen's edge. The sample will undergo a constant minor load of 44.5N. The Durometer tester quantifies the displacement of the indenter immediately following each indentation, and the apparatus automatically converts this result into a scale ranging from 0 to 100 units. The final hardness value will be determined by visually reading the analogue immediately after applying the load for 1 second $^{(13)}$.

2. Fourier Transform Infrared Spectroscopy:

The Fourier Transform Infrared Spectroscopy (FTIR) test was recruited to determine whether there was a chemical reaction or alteration following the inclusion of the additives utilized in this investigation ^(3,14). The FTIR analysis was carried out using the **IRAFFINITY-1S-FTIR** spectrophotometer (ShIMADZU, Japan). The analysis was performed at the Intravenous Solution Laboratory in Mosul. Iraq. The spectrophotometer had a resolution of 0.5 cm⁻¹ and was capable of measuring wavenumbers in the range of 7,800 to 350 cm⁻¹.

-Fourier Transform Infrared Spectroscopical Characterization of the prepared conjugated nanomaterials: With advancements in modern FTIR spectroscopy machines, we may now directly apply the sample (in powder form) for testing purposes. Twelve specimens were constructed, three specimens for each group (nystatin group, nano-nystatin group, MWCNTs group, multi-walled carbon nanotubes impregnated with Nystatin nanoparticles group).

- Fourier Transform Infrared Spectroscopy Test (FTIR) of MWCNTs-Nys- CHDR: Eight specimens were constructed, two specimens for each group. The dimensions of each specimen were $10 \times 10 \times 2 \pm 0.02$ mm, representing the length, width, and thickness accordingly. The specimens were then maintained in distilled water at a temperature of $37^{\circ}C \pm 1^{\circ}C$ for a period of 48 hours for conditioning, as described by Nandiyanto *et al.* (2019) and Urban *et al.* (2007) ^(3 & 15). In previous studies, the specimens were ground into a powder and mixed with potassium bromide salt, then compressed under pressure to form a pellet to be examined by FTIR spectroscopy ⁽¹⁶⁾. However, due to developments in FTIR spectroscopy technology, we are now able to test samples directly using the SHIMADZU IRAFFINITY-1S-FTIR spectrophotometer ⁽¹⁷⁾.

A normality test was applied to assess the data distribution, the Kolmogorov-Smirnov test showed that data of all variables was normally distributed (Table 1). Statistical analysis for all data was done by the SPSS system (version 28).

 Table (1): Kolmogorov-Smirnov test of data distribution normality.

	Kolmogorov-Smirnov ^a			
CHDR groups	Statistic	df	df Sig.	
Control	0.246	5	.200*	
MWCNTS-Nys 0.025%	0.229	5	.200*	
MWCNTS-Nys 0.05%	0.221	5	.200*	
MWCNTS-Nys 0.1%	0.231	5	.200*	
*This is a lower boun	nd of the true	signifi	cance.	
a. Lilliefors Signific	cance Correct	ion		

RESULTS

1. Surface hardness:

The mean and standard deviations for Shore D values of nonmodified (control) and modified chairside hard denture reliner (MWCNTs-Nys-CHDR at 0.025%, 0.05%, 0.1% wt) was presented in Figure (2). The modified CHDR exhibited the highest Shore D value at 0.1% wt, reaching 76.20. In comparison, the control group exhibited a lower Shore D value of 64.20.

The analysis of variance (Table 2) explored the impact of including MWCNTs-Nys on the shore D values of the modified CHDR. The results demonstrated statistically significant differences between the modified CHDR with varying concentrations and the control group, with a p-value of ≤ 0.05 . Duncan's multiple range test results, mean values, and standard deviations for the control and modified CHDR shore D. are displayed in Figure (2). The results indicate statistically significant differences between all modified groups and the control samples. There were no substantial differences in the shore D value among MWCNTs-Nys 0.025% and MWCNTs-Nys 0.05%.

Table (2)ANOVA of shore D comparisonbetween control and modified CHDR.

	Sum of Squares	df	Mean Square	F	Sig.
Between	383.600	3	127.867	64.743	.000
Groups					
Within	31.600	16	1.975		
Groups					
Total	415.200	19			



Figure 2: Mean, S.D, and Duncan's multiple range test for shore D of control and modified CHDR.

2. Fourier Transform Infrared Spectroscopical Characterization of the prepared conjugated nanomaterials and MWCNTs-Nys- CHDR.

- FTIR of Nystatin

The FTIR spectrum of pure nystatin powder is depicted in Figure (3) and exhibits all the distinctive peaks associated with nystatin⁽¹⁸⁾. It is known that nystatin has unique absorption bands around 1702 cm⁻¹. These bands are caused by the carbonyl group in the carboxylic acid stretching vibration. It also shows absorption bands at around 1576-1600 cm⁻¹, indicating the presence of double bonds (CH = CH), and at approximately 850 cm⁻¹, which corresponds to the bending vibration of (-CH) groups. Additionally, there are absorption peaks at approximately 1062 cm⁻¹, indicating the presence of hydroxyl groups, as well as peaks at 3415 cm⁻¹ (stretching vibration of O-H), 2924 cm⁻¹ (stretching vibration of C-H), and 2358 cm⁻¹ (presence of C=C groups) ^(15,19).

- FTIR of Nano- Nystatin:

The FTIR spectrum of nano-nystatin powder exhibits all the distinctive peaks observed in pure nystatin powder as shown in Figure (3) and in earlier research, but with minor shifts ⁽²⁰⁾.



Figure 3: The FTIR spectrum of (a) pure macro-Nystatin, (b) Nano-Nystatin powder.

- FTIR of Cooh-MWCNTs

The FTIR analysis of Cooh-MWCNTs (Figure 4) revealed the distinct stretching vibrations of O-H groups at a wavenumber of 3400 cm⁻¹. The appearance of a peak at 1730 cm⁻¹ in the spectra of the oxidized MWCNTs can be attributed to the elongation of the C=O bond in the carboxylic group (COOH) as a result of the oxidation process occurring on the surface of the oxidised MWCNTs. In addition, the presence of a band at 1650 cm⁻¹ signifies the existence of an amide carbonyl (C=O) stretching. The presence of a band at 1523 cm-1 corresponds to an N-H inplane vibration. The presence of the amide functional group is confirmed by Delfi et al. (2020)⁽²¹⁾. The existence of peaks at 2364 and 2342 cm⁻¹ corresponds to the elongation of (C=C) bonds, together with peaks at 2890 in addition to 2960 cm⁻¹ corresponding to the stretching of (C-H) bonds (22 & 23).

- FTIR of MWCNTs-Nano-nys

Figure (4) demonstrates that the major peaks observed in both Nano-Nystatin powder and Cooh-MWCNTs are also present in MWCNTs-Nano-nys. Multiple peaks within the range of 1200-1680 cm⁻¹, indicating the presence of an amide group ^(15,23). The observed prominent peak at 1650 cm⁻¹ corresponds to the C=O amide group exhibiting stretching vibrations present in MWCNT-COOH. Typically, it is observed within the spectral range of 1600–1700 cm^{-1 (23&} ²⁴⁾



Figure 4: The FTIR spectrum of (a) Cooh-MWCNTs powder, (b)MWCNTs-Nano-nys.

- Fourier Transform Infrared Spectroscopy Test (FTIR) of chairside hard denture reliner (MWCNTs-Nys- CHDR)

The FTIR of nonmodified (control) and modified chairside hard denture reliner (MWCNTs-Nys-CHDR) at 0.025%, 0.05%, 0.1% wt was demonstrated in Figure (5). Expressing that a major peak for the control chairside hard denture reliner was at (1723 cm⁻¹) confirming the presence of carbonyl groups (C=O) that typically exhibits a peak in the range of 1640-1850 cm⁻¹. And the absorption peak seen

around 1456 cm⁻¹were the stretching at vibrations of the carbon-carbon (C-C) bonds in the polymer backbone, which can appear as broad peaks in the range of 1400-1500 cm⁻¹, with a slight prominent peak at 1141 cm⁻¹ representing (C-O) bonds that increase in intensity with increasing additive's concentration. The absorption peak close to 1450 cm⁻¹ in an FTIR spectrum is commonly linked to the flexing vibration of the methyl (CH3) group. This peak is typically observed in organic compounds that contain methyl groups, such as alkyl chains or methyl substituents. also expressing another two sharp prominent peaks of C-H seen at (2852.72 and 2922.16 cm⁻¹) which have been identified in all groups ^(15,24). No new peaks were observed in the modified CHDR compared to the control CHDR, indicating that no chemical reaction developed between the chairside hard denture reliner and the MWCNTS-Nys applied at varied concentrations (0.025%, 0.05%, 0.1% wt). But, with the addition of MWCNTS-Nys and as the additive's concentration increases, there is a simple shifting of the amide group peak from (1725 cm⁻ ¹) at the control group into (1716.65 cm⁻¹) at the greatest concentration of the additive, which is 0.1% by weight.



Figure 5: FTIR of chairside hard denture reliner(a) control group (b) 0.025% wt group(c) 0.05% wt group(d) 0.1% wt group.

DISCUSSION

The utilization of engineered nanotechnologies enabled novel uses as drug carriers in dentistry which is rapidly growing ⁽⁹⁾. Nanonystatin exhibits enhanced antifungal efficacy in comparison to traditional nystatin. The studies conducted by Kassem et al. (2016), and Saleemi et al. (2020) ^(25,26) have highlighted the benefits of nanosizing nystatin and incorporating nystatin into multi-walled carbon nanotubes (MWCNTs) for achieving a continuous and sustained release of the drug from denture reliners, and have enabled the development of novel uses. Furthermore, by using this loading technique, we have also tried to enhance the hardness of the material without causing any chemical alterations.

- Hardness

The study was conducted to examine the surface hardness of a chairside hard denture reliner that had been modified. The modification involved incorporating multi-walled carbon nanotubes (MWCNTs) loaded with Nystatin nanoparticles at various concentrations (0.025%, 0.05%, and 0.1% by weight). Our modification will reduce the necessity for frequent disinfection, which is highly beneficial for patient acceptance and maintaining material hardness. This is particularly important as Tokuyama CHRL hardness has been observed to diminish after 30 days of repeated disinfection (27). The Shore D value served as a measure of surface hardness, with higher values denoting increased hardness. The control group, which was not subjected to any alteration, exhibited a Shore D value of 64.20. The groups that had been modified exhibited a progressive increase in Shore D values as the concentration of MWCNTs-Nys increased, ultimately reaching a peak value of 76.20 at a concentration of 0.1%. This aligns with the findings of Alraziqi and Mansoor (2020), and Mutahar et al. (2023) (28 & 29), who concluded that the hardness was enhanced by enhancing the concentration of MWCNTs. This enhancement can be attributed to the robust strength and Young's modulus of the MWCNT reinforcement, as well as the overlapping and stacking effect, which restricted the mobility of polymer molecules owing to the reduction in its free volume and molecular mobility which could result from upgrading internal adhesion (30) and the existence of a uniform dispersion of CNT within the materials can account for this phenomenon⁽³¹⁾.

Consequently, this led to an increase in the material's resistance to scratching, cutting, and plastic deformation.

The statistical analysis identified significant differences between the modified groups and the control group (p-value ≤ 0.05). In addition,

Duncan's multiple range test revealed statistically significant disparities between all altered groups and the control group. Nevertheless. there no statistically was significant variance in Shore D value found between the MWCNTs-Nys 0.025% and MWCNTs-Nys 0.05% groups. that concurs with previous research proving that the best enhancement occurs with the addition of not more than 0.1 % of MWCNTs (32). The results suggest that the addition of MWCNTs-Nys at different concentrations has a notable effect on the surface hardness of the chairside hard denture reliner. Higher concentrations of MWCNTs-Nys lead to enhanced hardness, which may enhance the durability and endurance of the denture reliner that is concurred with previous research applied on polymethyl methacrylate containing (MWCNTs) as materials for denture bases. (31,32 & 34), and for other polymers also studied by (Muñoz-Chilito et al., 2023) (35) stating that an increase in the degree of crystallinity of the polymer matrix promotes the stiffness or hardness of polymers.

-Fourier Transform Infrared Spectroscopy Test (FTIR)

Fourier Transform Infrared Spectroscopical assessment is important for the exploration of composite polymeric structures ⁽³⁶⁾. Since it

offers information about the interactions and complexation between the various constituents of the polymers. These chemical and/or physical interactions can induce variations in the vibrational modes of the polymer molecules ^(15,17,37). FTIR characterization of the prepared conjugated nanomaterials and MWCNTs-Nys-CHDR showed that:

- FTIR spectrum of nystatin and prepared nano nystatin (Figure 3) showed absorption bands that are characteristic of pure nystatin which concurred with another research, that proves to preserve the same chemical structure of the original nystatin ^(18 & 38).

Nanonystatin FTIR spectrum reveals fewer pronounced peaks, which can be attributed to the increased crystallization of the obtained nanonystatin powder and absorbance change in the 39). (25 & infrared area The increased crystallization of nanosized drugs was confirmed by the use of FTIR. It is commonly recognized in the field of drug formulation and delivery that decreasing the crystallinity of medication particles enhances their bioavailability. Enhancing the bioavailability of drug particles can be achieved by reducing their crystallinity, which in turn increases solubility, improves dissolution rate, and minimizes the likelihood of crystallization. These factors enhance drug absorption and increase the

proportion of the provided dose that is released, leading to enhanced therapeutic effectiveness ^(25, 39).

-FTIR of Cooh-MWCNTs (Figure 4) showed all the characteristic stretching vibrations of Cooh-MWCNTs and verified the existence of the amide functional group ⁽²¹⁾. Also, the presence of peaks at 2364 and 2342 cm⁻ ¹ related to stretched (C=C), in addition to peaks at 2890 and 2960 cm⁻¹ related to (C-H) stretching ^(22,23). To determine the surface functional groups of the oxidized carbon nanotubes (CNTs), Fourier Transform Utilizing infrared spectroscopic analysis, the presence of the C =O functionality in the COOH group was detected at a wavelength of 1730 cm⁻¹. This corresponds to the stretching vibration of the carboxylic groups, as reported by Nandiyanto et al. (2019) and Rong et al. (2010) (15,40). Evidence indicates that carboxylic acids have been generated on the nanotubes' surface to insure the surface functionalization of carbon nanotubes (CNTs) ⁽⁴⁰⁾. The study revealed a prominent peak at 1650 cm⁻¹, which was credited to the elongation vibrations of the C=O bond in the amide group of MWCNT-COOH. Typically, it is observed within the spectral range of 1600-1700 cm⁻¹. Hence, the existence of carbonyl groups can be attributed to this phenomenon, serving as evidence for the effective oxidation and functionalization of CNTs $^{(21,23)}$.

- FTIR of MWCNTs-Nano- Nys. The prominent peaks in Figure 4 illustrate that those detected in both nano-Nystatin powder and Cooh-MWCNTs are also evident in MWCNTs-Nano-Nys. The peaks manifest at certain wavenumbers: 1730 cm⁻¹, 1650 cm⁻¹, 1702 cm⁻¹, approximately 1576–1600 cm⁻¹, and about 850 cm⁻¹. Multiple peaks within the range of 1200-1680 cm⁻¹ suggest the existence of an amide group (15 & 23). The significant peak observed at 1650 cm⁻¹ is credited to the elongation vibrations of the C=O bond in the amide group found in MWCNT-COOH. Usually, it is detected in the spectral range of 1600-1700 cm⁻¹. According to the studies done by David et al. in 2021 and Uttekar et al. in 2016 (23,24), this demonstrates how the presence of the amide group facilitates successful drug conjugation. The presence of the nystatin drug loaded on multi-walled carbon nanotubes (MWCNTs) is characterized by a prominent and wide peak at around 3388 cm⁻¹. This peak signifies the simultaneous occurrence of stretching vibrations of N-H and O-H bonds (38)

- Fourier Transform Infrared Spectroscopy Test (FTIR) of chairside hard denture reliner (MWCNTs-Nys- CHDR).

The FTIR spectrum (Figure 5) of the control group showed absorption bands that are characteristic of the PEMA of the denture reliner. While the FTIR spectra of the experimental groups showed absorption bands that are characteristic to the incorporated MWCNTs-Nys in addition to that of CHDR (Figure 5). Indicating that it did not affect or interfere with the polymerization reaction of the polymer nor affect its chemistry, from which, one could realize that there was no chemical interaction. As the FTIR spectra of the experimental group showed the same FTIR absorption bands of the control group, but with slight peaks shifting in addition to new absorption peaks that are characteristics for the incorporated MWCNTs-Nys. The shift of absorption bands in the FTIR spectrum could be due to the physical bonding resulting from crosslinking among the bent polymeric networks ^{(40).} Shifting of amide group peaks at (1716.65 cm⁻¹), and increasing peak intensity is proof of efficient drug conjugation (drug loading), which increases drug stability, decreases toxicity, and ensures steady, sustained drug release from the hard reliner. The presence of C=C bonds in MWCNT is shown by the bands at around 1653, 2364, and 2342 cm⁻¹. This band sharpened in the case of nanohybrids and nanocomposites, which may be due to the combined effect of nystatin and MWCNT. Additional distinctive bands of nystatin were detected at 2852 and 2922 cm⁻¹, resulting from the symmetrical and asymmetrical vibrations of -CH2 groups. These bands are clearly visible in the case of the nanocomposite thermosets. All peaks showed increased intensity and sharpened with increasing additive concentration and no chemical interaction between nano additives and polymers as concurred with previous research ^{(5,} 39)

CONCLUSIONS

The findings of this experiment indicate that the bioactive alteration of CHDR resulted in a significant increase in hardness. without any chemical alterations as compared to the control group. Thus, it is essential to reject the null hypothesis.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Author contributions

R. R. A. studied conception and design, methodology, statistical analysis and interpretation of results, original draft manuscript preparation, writing - review & editing. M. M. S. supervised the work. All authors reviewed the results and approved the final version of the manuscript to be published.

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