

Research Article

Novel Bioactivation of Denture Liner and Its Effect on Tensile Bond Strength and Structural Morphology

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Abstract: Background: The biomedical sciences focusing on creating novel medication delivery systems for controlled drug release is fascinating, as nanotubes are specifically employed for drug delivery and the possible improvement of denture liner physical properties.

Aims: The research project aims to explore the tensile bond strength and structural morphology of Multiwalled carbon nanotubes (MWCNTs)-Nanostatin(Nys)-modified hard chairside denture liner following nanosizing nystatin macromolecules for conjugation with MWCNTs and incorporation into chairside hard denture reliners.

Methods: The chairside relining material was produced according to the manufacturer's standards, relining specimens were made with a quadrangular shape (43×10×10mm). Tensile bond strength was tested using an Instron Universal Testing Machine, while morphological observations of MWCNTs-Nys-CHDR were conducted by using a Scanning electron microscope and High-Resolution Transmission Electron Microscopy. The chairside hard denture reliner specimens were divided into two groups: the control group (5 specimens) and the modified chairside hard denture reliner (MWCNTs-Nys-CHDR at 0.025%, 0.05%, and 0.1% by weight, 5 specimens each).

Results: The bioactive alteration of CHDR led to enhanced tensile bond strength in comparison to the control group. The highest tensile bond strength values were at 0.1% wt.

Conclusions: The bioactive alteration of CHDR led to significant improvement in tensile bond strength. All of the specimens utilized in this investigation showed tensile bonding strength values suitable for clinical use, with no structural morphology changes of the bioactive modified denture reliner with Nano-nys conjugated to MWCNTs. It also reveals that MWCNTs preserve their original hollow long tubular form.

Keywords: Nano nystatin, Multiwalled carbon nanotubes, denture liners, tensile bond strength.

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Received: 27/08/2024

Accepted: 23/09/2024

DOI: <https://doi.org/10.53555/AJBR.v27i3.2130>

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Introduction

Denture-bearing zones altered significantly following tooth extraction, requiring the relining of removable prostheses to provide proper fit and support. Autopolymerizing reline resins enable dentists to immediately reline removable prostheses within the mouth, saving time while perfectly duplicating the denture base with oral soft tissue properties (Reis et al., 2011) However, These chairside relining materials, such as polymethyl methacrylate, nonetheless have worse physicochemical and mechanical qualities than heat-

polymerized denture base resins. Researchers are always studying different techniques to increase the quality of denture base materials and hard denture reliners while keeping the integrity of the materials' features (Leite et al., 2022). The strength of the connection between the denture relines and denture resin is critical. A poor link can lead to the collection of microorganisms, discolouration, and finally separation of the reline material (Awad et al., 2023)

Nanotechnology has become a valuable instrument in biomedicine, notably in the delivery of medications, as

evidenced by the utilization of nanotubes and nanoparticles. Multi-walled carbon Nanotubes (MWCNTs) were combined with PMMA to generate a nano additive that may efficiently kill microorganisms without the need for pharmaceuticals (Makvandi et al., 2020). New efforts have been conducted to load medications onto MWCNTs for regulated and long-term drug release. Nystatin, a common topical therapy for fungal infections, may reduce patient compliance due to its frequent administration (Shraddha et al., 2022). The goal of this study is to develop nystatin-loaded Multi-Walled Carbon Nanotube reliners that improve tensile bond strength while retaining structural morphology. The null hypothesis proposed that adding MWCNTs impregnated with Nystatin nanoparticles at concentrations of 0.025%, 0.05%, and 0.1% by weight to the Chairside Hard denture reliner (Rebase II) (CHDR) would have no impact on the tensile bond strength or structural morphology of the bioactively modified denture reliner.

Materials and Methods

Study settings: The College of Dentistry's research ethical committee at the University of Mosul in Iraq (UoM. Dent. 23/13) approved this study on September 4, 2023.

The nystatin macromolecules were reduced to nanosize (31.44 nm median particle size) (Aziz and Sadoon, 2024a) and coupled with MWCNTs to be loaded into CHDR at 0.025%, 0.05%, and 0.1% by weight. This bioactive modification of chairside hard denture reliner resulted in improved antifungal efficacy lasting for up to sixteen weeks due to prolonged drug release (Aziz and Sadoon, 2024a, 2024b), which is regarded as a promising technique to prevent or cure denture stomatitis.

Tensile bond strength of hard denture relining materials to the traditional heat-cured acrylic denture base resin

Twenty specimens for tensile bond strength were prepared (control group, 5 specimens) and MWCNTS-Nys-CHDR (0.025%, 0.05%, 0.1% by weight, 5 specimens for each group) that were of quadrangular shape (43×10×10 mm).

A wax block was prepared by investing a metal bar with 43mm×10mm×10mm dimensions in the silicon rubber base to create a mould for molten wax and after solidification of wax, it was removed and used as a model for acrylic resin mould fabrication (Koseoglu et al., 2023).

The specimen was fabricated by investing a block of wax (43mm×10mm×10mm) in dental stone to create moulds for two denture base acrylic resin specimens (20×10×10) and a metal spacer (3×10×10) for a hard reliner layer was placed between two wax blocks. After wax elimination, the acrylic resin was packed with the trail closure technique. The metal spacer for the hard reliner was removed and hard reliner

packing between the two acrylic resin pieces was carried out (Koseoglu et al., 2023).

Denture base specimens' production: A total of forty specimens of denture base specimens were prepared using heat-polymerized resin from Ivoclar Vivadent AG in Liechtenstein. Wax specimens from a putty elastomer mould with a quadrangular form (43×10×10 mm) were flaked using a traditional flasking procedure. After that, a coat of vaseline was put above the primary stratum of stone, followed by another layer of stone mixture on the superior half, which covered the specimens. The flask's lid was then positioned to allow any extra gypsum to escape out. After the gypsum had fully set, the flask was immersed in boiling water for 4 to 6 minutes for wax clearing. After applying a separating fluid to the impressed gypsum (Ivoclar Vivadent AG, Liechtenstein), a heat-polymerizing resin (Triplex heat polymerized denture base resin, Ivoclar Vivadent AG, Liechtenstein) was produced and put into the flask with a powder/liquid ratio of 23.4/10 g/mL. The set was polymerized using a hydraulic system that met the manufacturer's specifications (heating to 100°C and boiling for 45 minutes). After being taken from the water, the set was allowed to cool to ambient temperature before the specimens were retrieved. Curing and deflasking are performed as described above (Koseoglu et al., 2023).

Relining procedure: The relining process involved putting the mixed material above the denture base cube, as directed by the manufacturer (mixing the powder and liquid completely for about 30 seconds with a spatula, scraping the sides and bottom of the mixing pad to ensure complete mixing). Apply the combined material to the denture's surface in a thin, homogeneous layer, ensuring that there is enough material to offer proper support and stability. Allow the Tokuyama Rebase II Fast material to cure for approximately 5 minutes. After curing, remove any excess material with a bur or sharp instrument (Awad et al., 2023).

After polymerization, all the samples were removed from the moulds and manually polished with 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL) to remove irregularities, then stored in distilled water for 24 hours at 37°C before being tested by Instron Universal Testing Machine (GESTER International Co., LTD, Quanzhou, China) with 1kN load at a crosshead speed of 5 mm/min constant velocity until failure has occurred (Awad et al., 2023).

The maximum tensile load before failure has been evaluated and tensile bond strength was computed using the following equation (Koseoglu et al., 2023):

$$\text{Tensile bond strength} = \frac{\text{Maximum load (N) at failure point}}{\text{cross-sectional area (mm) of the interface}}$$

The cross-sectional area (10×10 mm) was 100 mm² for all denture base specimens (Koseoglu et al., 2023). Tensile strength elongation curves were documented by the computer software of a universal testing machine for each sample (Figure 1).

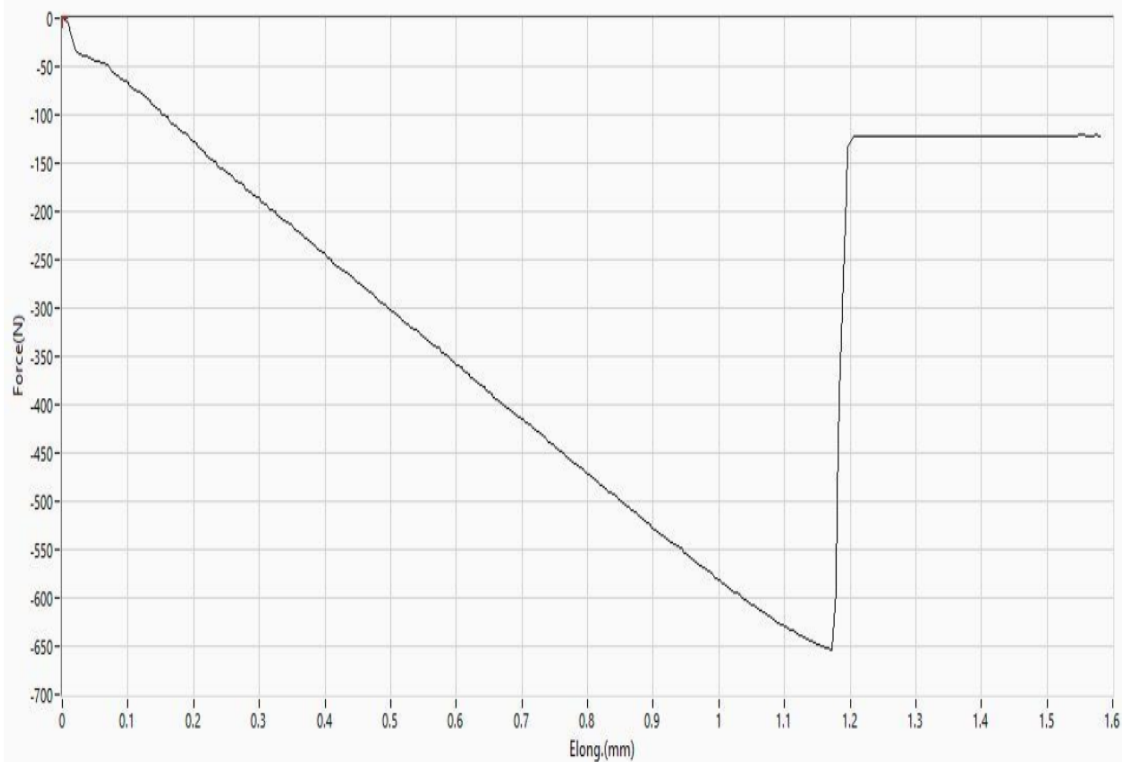


Figure 1. Tensile bond strength elongation curve

Tensile bond strength failure mode evaluation: The failure mode on the separated surfaces was evaluated by two observers using a stereomicroscope and classified as adhesive, cohesive, or mixed. Failures were classified as adhesive if they took place between the relined resin and the denture base resin, and cohesive if the fracture occurred only inside one of the resins. If the fracture occurred at the interface of the two resins yet contained traces of relined resin, it was considered to be mixed. The failure type percentage was estimated with the following equation: (failure type number/total number) $\times 100\%$ (Awad et al., 2023; Koseoglu et al., 2023).

Morphological Observations of MWCNTs-Nys- CHDR by using Scanning electron microscope (SEM) and High-Resolution Transmission Electron Microscopy (HRTEM): The surface morphology of SEM image of the control CHDR specimens and the modified CHDR specimens was studied by scanning electron microscope (TESCAN Mira3, French) at 50 and 100 kx magnification, that can provide information about the morphology, size, and the distribution of MWCNTs-Nano-nys in CHDR specimens (David et al., 2021; David et al., 2021a). One specimen for each group with dimensions of $10 \times 10 \times 2 \pm 0.02$ mm (length, width and thickness respectively) was prepared according to instrument specifications. All specimens were coated with a 20 μ m gold layer by a sputter coating machine to be electroconductive specimens before subjecting them to imaging by SEM with an accelerating voltage of 15 keV at 5 and 100 kx magnification (Muralidhar et al., 2012).

The internal structure of MWCNT-Nys was studied by TEM: Transmission Electron Microscopy (HRTEM) is an advanced imaging technique used to study the structure and properties of materials at the nano and atomic scale. It provides detailed information about the crystal structure, morphology, and composition of materials (Andrian et al., 2021). It helps to prove that the true drug conjugation is represented by the attached nystatin particles to the surface, edge, and inside MWCNTs.

Statistical analysis: Data expressed as mean \pm SD. In the one-way analysis of variance (ANOVA), the tensile bond strength (MPa) of the modified chairside hard denture reliner was substantially in comparison to that of the control group. Duncan's multiple range test, mean, and standard deviation for control and modified CHDR tensile bond strength. The p-value less than 0.05 is considered as significant. Statistical analysis for all data was done by the SPSS system (version 28)

Results

Tensile bond strength of hard denture relining materials to the traditional heat-cured acrylic denture base resin: The descriptive analysis of tensile bond strength values for nonmodified (control) and modified reliner (MWCNTs-Nys-CHDR at 0.025%, 0.05%, 0.1% wt), with a highest value showed at 0.1 %wt of the modified chairside hard denture reliner (11.39 MPa), while lower value presented at control group (2.246 MPa) (Table 1).

Table 1. Tensile bond strength (MPa) for control and modified CHDR

| CHDR groups (n=5 each group) | Mean±SD |
|------------------------------|----------|
| Control | 2.25±0.2 |
| MWCNTs-Nys 0.025% | 4.3±0.83 |
| MWCNTs-Nys 0.05% | 6.4±1.2 |
| MWCNTs-Nys 0.1% | 11.4±1.9 |
| Total (n=20) | 6.07±3.7 |

The one-way analysis of variance (ANOVA), the tensile bond strength (MPa) of the modified chairside hard denture reliner was substantially in comparison to that of the control group at $p \leq 0.05$ (Table 2).

Table 2. Tensile bond strength comparison between control and modified CHDR

| Source of variance | Sum of Squares | df | Mean Square | F | P |
|--------------------|----------------|----|-------------|-------|--------|
| Between Groups | 231.282 | 3 | 77.094 | 55.43 | 0.0001 |
| Within Groups | 22.254 | 16 | 1.391 | | |
| Total | 253.536 | 19 | | | |

One way ANOVA

Duncan's multiple range test, mean, and SD for control and modified CHDR tensile bond strength, expressing statistically significant differences between all groups (the control and modified groups). A significant increase in tensile bond strength value for all modified groups occurred and increased as the concentration of additive was increased.

(0.025%, 0.05%, 0.1% wt). Adhesive failure was predominant for the tensile bond strength for the control group, while, all the modified CHDR (0.025%, 0.05%, 0.1% wt) showed mixed mode of failure for the tensile bond strength. Therefore, it supposes that the tensile bond strength mode of failure is greatly dependent on the addition of MWCNTs-Nys nanoparticles, and their addition concentration would affect the tensile bond strength values.

Failure Analysis of the Chairside Hard Denture Reliner

Table (3) shows the percentage of the mode of failure for the tensile bond strength for control and modified CHDR

Table 3. Tensile bond strength mode of failure for the control and modified CHDR groups.

| Study groups | No. | Failure % | |
|--------------------------|-----|------------|--------|
| | | Adhesive % | Mixed% |
| Control | 5 | 60 | 40 |
| MWCNTs-Nys 0.025% | 5 | 0 | 100 |
| MWCNTs-Nys 0.05% | 5 | 0 | 100 |
| MWCNTs-Nys 0.1% | 5 | 0 | 100 |

Morphological Observations of MWCNTs-Nano-nys by using SEM

The surface structure of MWCNTs-Nano-nys was studied by scanning electron microscope. SEM image of the MWCNTs-Nano-nys specimens at 100 and 50 kx magnification showed

the morphology, size, and random distribution of MWCNTs-Nano-nys specimens, the size of nystatin nanoparticles attached to the surface and edges of the MWCN tubes(D1=40.05, D2= 46.06, D3= 43.42 nm) (Figure 2).

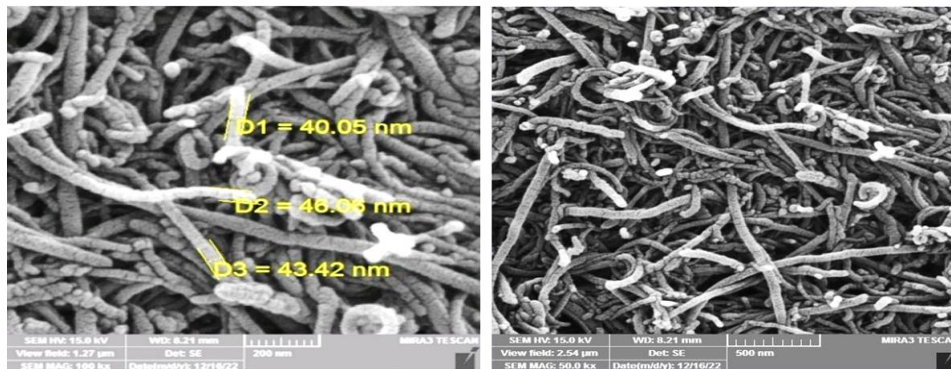


Figure 2. Morphological Observations of MWCNTs-Nano-nys by using (SEM). SEM image of MWCNTs-Nano-nys specimen at 100kx & 50kx magnification.

Transmission Electron Microscopy of MWCNTs-Nano-nys (TEM): Figure (3a) reveals the TEM of Nano-nys -MWCNTs specimen. Figure (3b) reveals the TEM of Nano-nys outside or at the surface of the MWCNTs, Figure (3c) reveals the TEM of Nano-nys inside the MWCNTs, Figure

(3d) reveals the TEM of Nano-nys (inside, at the surface and the edge of MWCNTs). TEM also reveals that MWCNTs preserve their original hollow long tubular form after their chemical preparation and nystatin loading.

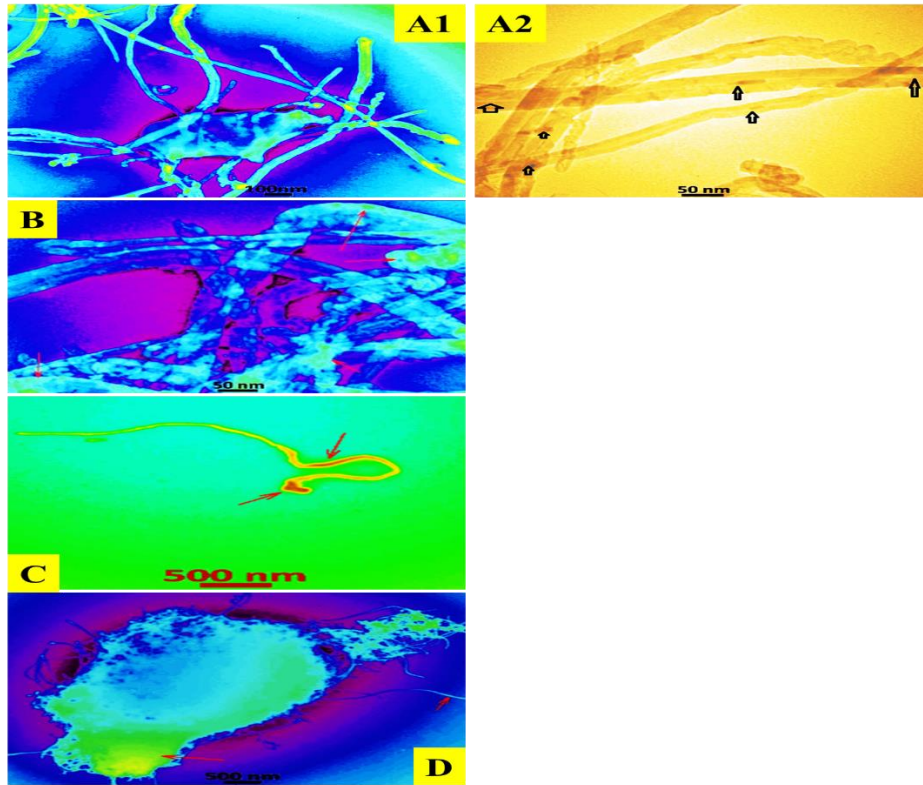


Figure 3. Transmission Electron Microscopy of MWCNTs-Nano-nys (TEM). (A) TEM of Nano-nys-MWCNTs (B) TEM of Nano-nys at the surface of MWCNTs, (C) Inside the MWCNTs, and (D) inside, at the surface and the edge of MWCNTs.

Morphological Observations of chairside hard denture reliner by using (SEM): The SEM image at 5 and 100 kx magnification of the control CHDR specimens shown in Figure (4a) demonstrated no NP presence, and the modified

CHDR specimens showed the morphology and the random distribution of Nano-nys -MWCNTs within the different samples without aggregation of the nanotubes (figure 4b – 4d).

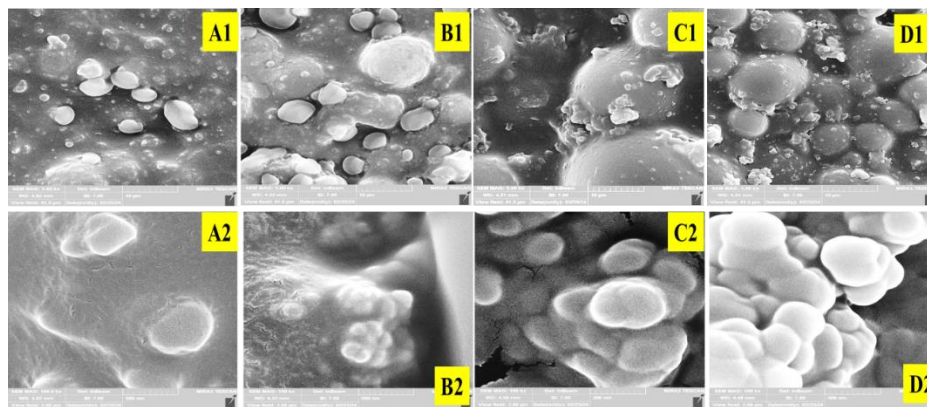


Figure 4. Morphological Observations of of chairside hard denture reliner by using (SEM). (A)SEM image of the control CHDR specimens at 5kx (A1) and (A2)100 kx magnification. (B) SEM image of the modified chairside denture reliner at 0.025% wt at 5kx (B1) and (B2)100 kx magnification. (C) SEM image of the modified chairside hard denture reliner at 0.05% wt at 5kx (C1) and (C2)100 kx magnification. (D)SEM image of the modified chairside hard denture reliner at 0.1% wt at 5kx (D1) and (D2)100 kx magnification.

Discussion

A medication delivery system based on acrylic resin hard reline loaded with MWCNTS-Nys is proposed for the future prophylaxis and medical treatment of *Candida albicans*-related denture stomatitis. It eliminates the need for patient compliance and lowers the requirement for clinical monitoring. Additionally, it may provide less dangers than standard therapies involving topical or systemic antifungals. Moreover, it can improve denture stability and retention through a simple and inexpensive therapeutic procedure. Our previous analysis revealed that our technique may be a viable choice for achieving that target. Furthermore, the reline resin showed antibacterial activity against *C. albicans*. It also offers a sixteen-week drug release of therapeutic doses (Aziz and Sadoon, 2024a, 2024b). As a result, it could be an effective technique for preventing or treating denture stomatitis.

Making a permanent connection between the relining material and the acrylic denture resin is an absolute requirement for proper denture relining. Three tests are usually employed to assess the strength of this joint: peel, shear, and tensile. The most common and reliable approach is the tensile strength test, which involves stretching the sample axially at a steady speed at room temperature until it fully breaks. In compliance with the guidelines of ISO 10139-2 (ISO, 2009; Vuksic et al., 2023)

The results of tensile bond strength (figure 3, table 1 and 2), obviously showed statistically significant tensile bond strength improving for modified CHDR with different concentrations in contrast to the control group. With a peak value shown at 0.1 %wt of the modified CHDR (11.39 MPa), while the lowest value was presented in the control group (2.246 MPa). This aligns with the findings of other researchers (Awad et al., 2023; Mahmood, 2015; Somkuwar et al., 2017), concluding that the tensile bond strength was enhanced by increasing the concentration of MWCNTs and that MWCNTs might serve as an excellent reinforcing material for denture base resins. This enhancement can be because the carbon matrix created by CNTs and PMMA was quite big, with a stronger link, which improved the characteristics. Incorporating CNTs with PMMA resins enhances prosthesis strength and resistance to masticatory stresses (Somkuwar et al., 2017; Turagam & Prasad Mudrakola, 2013). Also, it prevents polymerization shrinkage and dimensional changes in the resin, allowing for better adaptability of denture bases. It has already been established that adding various amounts of CNTs to acrylic resin enhances flexural strength, impact strength, and polymerization shrinkage (Turagam & Prasad Mudrakola, 2013), the result is similar to that with similar addition of 0.025% and 0.05% MWCNTs to heat cured acrylic (Mahmood, 2015).

This increase in the tensile bond strength for the modified liner with NPs may be due to an increase in the interfacial surface area so that the force requests to destroy the liner also increased, adding Nano filler will form a network of 3-dimensions of PEMA and Nanoparticles lead to improve the crystallinity of amorphous resin (Hasanen A. Alnamel, 2020). The low concentration of very small nano-size NPs used may have led to less tendency for agglomeration within the matrix, or possibly the added NPs may have increased the surface area of adhesion between the acrylic resin and the denture liner

(Abdelrahman et al., 2020). This result may be due to good diffusion of NPs in the polymer matrix with strong inter-atomic ionic bonding by the van der Waals forces between the nanoparticles and the polymer matrix, which causes a growth in the polymer chain's cross-linking with restriction of the polymer mobility and dense polymer matrix composite (Sun et al., 2021).

Adhesive failure was predominant for the tensile bond strength for the control group, while, all the modified CHDR (0.025%, 0.05%, 0.1% wt) showed the mixed mode of failure for the tensile bond strength. Therefore, it supposes that the tensile bond strength mode of failure is greatly dependent on the addition of MWCNTS-Nys nanoparticles, and their addition concentration would affect the tensile bond strength enhancement. It should be pointed out that, when adhesive failures are detected, the connection created by the bonding agent is to be considered as the weakest part of the connection; when dealing with mixed failures, connection strength and lining material are comparable; for cohesive failures, it may identify only the strength of the material itself is, in fact, lower than the strength of the interface layer formed by the bonding; thus, in practice, the mixed and cohesive failures must be considered especially promising (Jabłońska-Stencel et al., 2018).

This result agrees with Eurwongpanich et al. (2020) which found that the mixed mode of failure increased with nanomodified liners (Santi et al., 2020). And concurred with the fact that Mixed failures were found mostly in the specimens relined to (Tokuyama Rebase ii) (Awad et al., 2023). This means that this bioactive modification enhanced the cold-cured reliner's strength to be nearly equal to that of heat-cured acrylic denture base (Somkuwar et al., 2017), which shifts the majority of a cohesive failure that occurred in previous research (Awad et al., 2023; Koseoglu et al., 2023; Vuksic et al., 2023) into a mixed failure type, while adding nystatin to denture liner alone without CNTs didn't improve tensile strength and results in predominantly cohesive failure type (Shaikh et al., 2021).

The morphological observations of MWCNTs-Nano-nys were studied by scanning electron microscope. SEM image of the MWCNTs-Nano-nys specimens at 100 & 50 kx magnification showed the morphology and size, and it shows the random distribution of Nano-nys throughout the MWCNTs-Nano-nys powder specimens, which agrees with previous research (Ahmad et al., 2020; David, Ion, et al., 2021b), figure (4) also shows the size of nystatin nanoparticles attached to the surface and edges of the MWCN tubes (D1=40.05, D2=46.06, D3=43.42 nm), that agrees with previous researches (Misra & Pathak, 2020).

Transmission Electron Microscopy (TEM) is a technique for visualizing the interior structure of solids utilizing a beam of high-energy electrons sent through the solid substance (Rabiei et al., 2020).

Figure (5a-5d) for the transmission electron microscopy of Nano-nys-MWCNTs specimen, reveals Nano-nys at the surface of the MWCNTs, inside the MWCNTs, and the edge of MWCNTs. It also reveals that MWCNTs preserve their original hollow long tubular form after their chemical preparation and nystatin loading, as agreed with other researchers in relation to maintaining the patent tubular

structure of MWCNTs after chemical activation (Hassani et al., 2022; Jhansi et al., 2023). The surface morphology of CHDR incorporated with MWCNTS-Nys- CHDR was studied by scanning electron microscope. SEM image shown in Figure (6a-6d) at 5&100 kx magnification of the control CHDR specimens demonstrated no NP presence, and the modified CHDR specimens showed the morphology and the random distribution of Nano-nys - MWCNTs throughout the specimens without aggregation of the nanotubes, that agrees with previous researches (Ahmad et al., 2020; David, Ion, et al., 2021b).

Conclusions

The findings of this experiment indicate that the bioactive alteration of CHDR resulted in a significant improvement in tensile bond strength. All of the specimens utilized in this investigation showed tensile bonding strength values suitable for clinical use, the cold-cured reliner's strength enhanced to be nearly equal to that of heat heat-cured acrylic denture base, which shifts the majority of failures into a mixed failure type, with no structural morphology changes of the bioactively modified denture reliner with Nano-nys conjugated to MWCNTs. It also reveals that MWCNTs preserve their original hollow long tubular form.

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