

Article

Synthesis of Core–Shell Chitosan–TiO₂ Nanoparticles and Its Impact on *Candida albicans* Biofilm Inhibition on 3D-Printed Denture Base Resins: An In Vitro Study

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Abstract

Objective: This study aimed to obtain a core–shell chitosan–TiO₂ nanoparticle and to investigate its ability to inhibit *Candida albicans* biofilm formation when added to 3D-printed polymethyl methacrylate (PMMA) denture base resins. **Materials and Methods:** Ionic gelation was employed to prepare and characterize the nanoparticle, and Atomic Force Microscopy (AFM), Field Emission Scanning Electron Microscopy (FE-SEM), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray diffraction were used to identify the structure and morphology. Nanoparticle was added to 3D-printed denture resins at four different weight percentages (0.25%, 0.5%, 0.75%, and 1%) and antibiofilm activity was determined by carrying out Colony Forming Unit (CFU) counts after exposure to *C. albicans*. **Results:** The 0.25 wt.% chitosan–TiO₂ group exhibited a significant reduction in colony-forming units (CFUs) compared to the control ($p < 0.05$). Although higher nanoparticle concentrations showed improved biofilm formation, this was most likely caused by nanoparticle aggregation, which interfered with surface homogeneity and biofilm resistance. **Conclusions:** Incorporating a 0.25 wt.% core–shell chitosan–TiO₂ nanoparticle into 3D-printed denture base resin markedly improves its antibiofilm activity against *Candida albicans* while maintaining the material's integrity.

Keywords: core–shell chitosan–TiO₂; nanocomposite; *Candida albicans*; antibiofilm; 3D-printed denture base resins



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1. Introduction

In prosthetic dentistry, polymethylmethacrylate (PMMA) has long been the most commonly used foundation material for dentures and remains one of the most reliable options when modern approaches are not feasible. A variety of reinforcing techniques, including wires, nanoparticles, meshes, and fibers, have been researched and documented to remedy physical deficiencies caused by cyclic stress and unanticipated circumstances [1]. With advances in technology, there has been a rise in new computerized techniques for the fabrication of dental prostheses. Photocuring 3D printing represents the foundational technology in the realm of 3D printing, utilizing a photopolymerization process, with photosensitive liquid resin serving as the main printing material [2].

Consequently, it offers the advantage of producing smaller items with exceptional accuracy, capable of producing objects as tiny as 50 µm [3,4]. Due to this method's fast polymerization rate and high precision, models can be rapidly printed [5]. Furthermore,

it eliminates the need for many laboratory steps of molding and the long process of traditionally heat-curing acrylic resin prostheses [6]. Despite the many benefits of 3D printers, resin has inferior properties compared to other traditional materials such as heat-cured acrylic and CAD-CAM milling resin [7,8]. Due to the numerous benefits of nanoparticles, including their resistance to wear and tear, as well as their anti-corrosion properties, they are increasingly utilized in the field of materials science. Modifying the size of the filler enhances the characteristics of the material [9].

Two of the nanoparticles used in dental materials are chitosan and titanium dioxide (TiO_2). Titanium dioxide (TiO_2) is well known for its biocompatibility, non-toxicity, corrosion resistance, chemical stability, high strength, and high refractive index, as well as remarkable antimicrobial properties [10,11].

Chitosan is a naturally occurring polysaccharide that has superior biodegradability and exhibits good compatibility with biological systems. Chitin is the main structural polysaccharide of crustacean shells. It is highly crystalline and insoluble in most solvents. Chitosan, obtained by heterogeneous alkaline deacetylation of chitin, becomes soluble in dilute acidic aqueous solutions due to protonation of its amino groups; Therefore, chitosan can be obtained from renewable and sustainable marine waste sources, such as crustacean shells, and is environmentally advantageous due to its biodegradability into non-toxic saccharide products [12]. Importantly, chitosan demonstrates antibacterial potential owing to its electrostatic interaction with bacteria. Chitosan exhibits antimicrobial activity that is strongly dependent on environmental pH. Under acidic or mildly acidic conditions, protonation of its amino groups (NH_3^+) enables electrostatic interactions with negatively charged microbial cell walls, a similar interaction underlies its antifungal activity against residues on the fungal cell wall [13,14]. The reactive functional groups in chitosan enable diverse chemical modifications, such as using acrylic acid as a coupling agent to interact with chitosan's cross-linking intermediates, thereby inducing cross-reactions that generate new chemical bonds [15,16].

Nonetheless, the CS- TiO_2 composite exhibits great potential applications including antimicrobial activity against bacteria and fungi; UV-barrier properties when it is used for packaging and textile purposes; environmental applications for removal of heavy metal ions and degradation of diverse water pollutants; biomedical applications as a wound-healing material, drug delivery system, or by the development of biosensors. Furthermore, no cytotoxic effects of CS- TiO_2 have been reported on different cell lines, which supports its use for food and biomedical applications. Moreover, CS- TiO_2 has also been used as an anti-corrosive material [17].

The hybrid composite of CS- TiO_2 has been extensively utilized in numerous technical applications because of its potential advantages. These applications range from acting as antimicrobial agents to packaging materials and producing films or coatings to preserve food. Furthermore, this hybrid composite has proven useful in promoting wound healing and skin regeneration, detecting glucose and alpha-fetoprotein, and even degrading water pollutants [17].

The oral cavity's most prevalent fungus, and the main cause of oral candidiasis, is *Candida albicans*. In addition to causing denture liner degradation and subsequent tissue irritation, fungal growth on dental restorations is also responsible for denture stomatitis, a condition that affects more than half of patients who wear dentures. Moreover, *C. albicans* has been described as an opportunistic species in periodontal and peri-implant lesions [18,19].

The aim of this study is to investigate the potential of a core-shell chitosan- TiO_2 nanoparticle ($\text{TiO}_2@\text{CS}$), when incorporated into 3D-printed denture base resin, to prevent the formation and adhesion of biofilm on denture base surfaces. To our knowledge, this

study is the first to examine the effects of a core–shell chitosan–TiO₂ nanoparticle on the growth and adherence of *Candida albicans* biofilm when incorporated into denture base resin.

2. Materials and Methods

2.1. Materials

Titanium dioxide nano-powder (Cat. No. 718467, CAS No. 13463-67-7), with a 21 nm primary particle size (TEM) and a $\geq 99.5\%$ trace metals basis, and low-molecular-weight chitosan powder (Cat. No. 448869, CAS No. 9012-76-4) were obtained from Sigma-Aldrich, St. Louis, MO, USA; acetic acid was obtained from Merck, Rahway, NJ, USA (Cat. No. 100063); 25% NH₄OH (Ammonium Hydroxide, EMSURE[®] ISO, Reag. Ph Eur, Darmstadt, Germany), distilled water, polymethyl methacrylate (PMMA) NextDent Denture 3D+ (Classic Pink) were purchased from NextDent (Soesterberg, The Netherlands); and Sabouraud Dextrose Agar (SDA, REF 610103 Liofilchem R© srl, Roseto, Italy), Sabouraud Dextrose Broth (SDB, REF MH033 HiMEDIA Laboratories, Maharashtra, India), absolute ethanol (99.8%, $M_w = 46.07 \text{ g mol}^{-1}$), and normal saline were obtained from Merck. The chemicals and materials utilized in this study were obtained at standard levels and employed without further purification.

2.2. Pilot Study

A pilot study was carried out to evaluate the process of integrating core–shell chitosan–TiO₂ nanoparticles into the PMMA resin, optimize the fabrication parameters, establish printing orientation, and determine the appropriate dilution factor for colony-forming unit (CFU) assays. The synthesized core–shell chitosan–TiO₂ nanoparticle was then incorporated into the resin at four different weight concentrations, 0.25 wt.%, 0.5 wt.%, 0.75 wt.%, and 1.0 wt.%, corresponding to nanoparticle masses of 0.3 g, 0.6 g, 0.9 g, and 1.2 g per 100 cm³ of resin, respectively, as shown in Table 1.

Table 1. Weight percentages of different combinations of core–shell chitosan–TiO₂ NPs in a 3D acrylic resin volume of 100 cm³, and the total volume of 3D-printed resin with the mass of chitosan–TiO₂ NPs.

3D-Printed Resin Volume (cm ³)	Density (1.2) Weight (g)	Nanoparticles Percentage	Grams/cm ³ Nanoparticles	Total (3D-Printed Resin)
100	120	0.25	0.3	119.7
100	120	0.5	0.6	119.4
100	120	0.75	0.9	119.1
100	120	1	1.2	118.8

Published studies investigating the incorporation of antimicrobial nanoparticles such as TiO₂, Ag, and other metal oxides into acrylic and 3D-printed denture resins have most commonly evaluated nanoparticle loadings in the low weight percent range, often below 3 wt.%, with many studies focusing specifically on concentrations ≤ 1 wt.% to balance antimicrobial efficacy with physical and mechanical integrity of the polymer matrix. A systematic meta-analysis of nanoparticle-modified dental acrylic resins reported that low concentrations such as ≤ 0.25 wt.% TiO₂ and similar levels of other nanoparticles could enhance antimicrobial activity without adversely affecting physico-mechanical properties, supporting the use of low to moderate nanoparticle loadings in this range [20]. Additionally, studies on PMMA and TiO₂ nanocomposites have identified ranges such as 0.2–2.5 wt.% that exhibit antimicrobial effects against *Candida* species and bacterial pathogens, while higher concentrations may lead to aggregation and compromised material properties [21,22].

2.3. Synthesis of Core–Shell Chitosan–TiO₂ Nanoparticle

A solution was prepared by adding 10 g of titanium dioxide nano-powder to 1000 mL of 1% (v/v) acetic acid, followed by stirring for 90 min at 50 °C using a magnetic stirrer, then ultrasonication for 2 min (Q700 Sonicator; Qsonica LLC, Newtown, CT, USA), where the surface of TiO₂ formed Ti⁴⁺ ions, previous studies have reported that TiO₂ modified or immobilized using organic acids, such as citric acid, can be employed as nanohybrid coatings on medical devices to improve biocompatibility, inhibit thrombosis, and promote re-endothelialization [23]. However, in the present study, acetic acid was exclusively used as the solubilizing agent, and no citric acid modification was performed. Meanwhile, another solution was prepared by adding 10 g of low-molecular-weight chitosan to 1000 mL of 1% (v/v) acetic acid, which was also stirred for 90 min at 50 °C with a magnetic stirrer and then ultrasonicated for 2 min.

Both solutions (1000 mL of TiO₂ and 1000 mL of CS) were mixed; the TiO₂ solution was added to the chitosan (CS) solution at room temperature under continuous magnetic stirring at 150 rpm to ensure uniform dispersion until a homogeneous white solution was obtained [24]. After that, the temperature of the mixture increased to 50 °C, and 25% NH₄OH Ammonium Hydroxide solution was added drop-by-drop to the mixture under continuous stirring until the solution pH reached 10. The resulting precipitate was heated to 80 °C for 3 h. Subsequently, a Büchner funnel was employed to filter the mixture, and the filtrate was rinsed three times with extra-distilled water until it reached a pH of 7. The washed precipitate was dried overnight in a vacuum oven set to 60 °C, after which it was crushed using a mortar to obtain a fine powder [24].

2.4. Mixing the Nanoparticles with the Denture Base Resin

Dental resin (PMMA) NextDent Denture 3D+ (Classic Pink) was manually mixed for 5 min to ensure proper integration of its components, following the manufacturer's guidelines. Subsequently, the synthesized hybrid nanoparticle was incorporated into 100 mL of NextDent resin in four distinct concentrations (0.25 wt.%, 0.5 wt.%, 0.75 wt.%, and 1 wt.%), as shown in Table 1. This mixture was manually stirred with a glass rod for 1 min, followed by ultrasonication for 2 min and vacuum treatment for 5 min to eliminate excess air bubbles and enhance the bonding between the nanoparticle and the resin [25].

The mixture was subjected to an extra minute of ultrasonication followed by 5 min of vacuum treatment to achieve optimal dispersion. While mixing the 3D printing resin with the nanoparticle, aluminum foil was utilized to protect it from ambient light, preventing premature polymerization.

2.5. Sample Preparation

Six groups ($n = 10$; 60 specimens in total with positive and negative controls) of disk-shaped specimens were prepared (10 mm in diameter, 2 mm in thickness) as shown in Figures 1 and 2a,b.

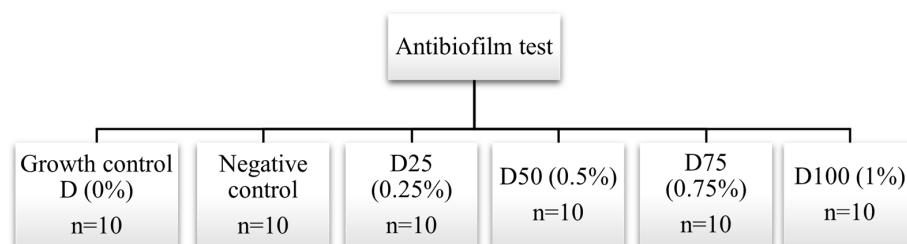


Figure 1. Study design showing the antibiofilm test groups, including the growth control, negative control, and four experimental concentrations (D25, D50, D75, and D100).

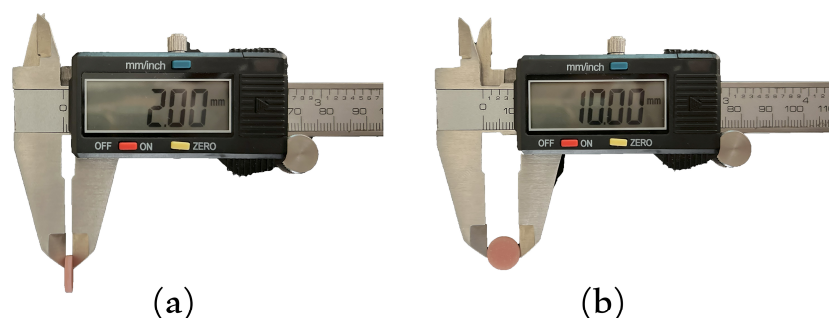


Figure 2. Digital caliper measurements of the 3D-printed disk-shaped specimen confirming standardized dimensions: 2.00 mm in thickness (a) and 10.00 mm in diameter (b), consistent with ISO 20795 specifications.

The manufacturer's instructions were carefully followed in the fabrication of all specimens, which were then assessed for consistency with the ISO specifications prior to testing [26]. The sample design was created utilizing 3D computer-aided design software (Blender for Dental v3.6.0; Blender Foundation, Amsterdam, The Netherlands), then the information was exported in Standard Tessellation Language (STL). The STL file was brought into the RayWare 3D printer software (v2.9.1; SprintRay Inc., Los Angeles, CA, USA) where it was sliced and placed on the build platform.

The synthesized mixture was then introduced into the 3D printing device's tank, with the necessary parameters configured for NextDent resin. Concurrently, the STL file was uploaded and methodically arranged in the software at (0) degrees without support, to optimize printing time and prevent nanoparticle precipitation. The fabrication process was conducted at a layer thickness resolution of 100 μm , as determined in the pilot study.

The resin samples were fabricated using the digital light processing 3D printing method (SprintRay Pro S Dental 3D Printer—Los Angeles, CA, USA); an LED light source with a wavelength of 405 nm powers the printer. For this specific resin, the RayWare software's default exposure time was used during printing. After printing, the resin samples were scraped off the build platform with a removal tool and washed for 10 min in 99% isopropyl alcohol in the washing machine (Pro Wash/Dry; SprintRay Inc., Los Angeles, CA, USA).

The fabricated materials were left to air dry for 10 min to evaporate the excess ethanol following the manufacturer's guidelines. The samples were then placed in a post-curing device (ProCure; SprintRay Inc., Los Angeles, CA, USA) with 405 nm LED arrays and a 360° reflecting interior for 6 min. After the printing and curing process, the specimens were finished utilizing silicon carbide grinding papers (1000, 1500, and 2000 grit FEPA) and washed with water, then polished with a polishing paste for 15 s on each side (universal polishing paste (High-luster), Renfert GmbH, Hilzingen, Germany) [27,28]. All samples were kept in water for seven days prior to testing. All resin samples were prepared, finished, and polished by a single operator to reduce variability. The final sample dimensions were verified using a digital caliper with ± 0.1 mm accuracy. Each group was coded as shown in Table 2.

Table 2. Specimen grouping and coding and the material descriptions of each group.

Group	Code	Material Description	N
Growth control (D0%)	D	3D-printed denture base resin without addition of nanoparticle	10
Negative Control	NC	3D-printed denture base resin, uninoculated sterility control excluded from analysis	10
D25 (0.25%)	D25	Denture base resin with 0.25 wt.% core-shell chitosan-TiO ₂ nanoparticle	10
D50 (0.50%)	D50	Denture base resin with 0.50 wt.% core-shell chitosan-TiO ₂ nanoparticle	10
D75 (0.75%)	D75	Denture base resin with 0.75 wt.% core-shell chitosan-TiO ₂ nanoparticle	10
D100 (1.00%)	D100	Denture base resin with 1.00 wt.% core-shell chitosan-TiO ₂ nanoparticle	10

2.6. Characterization

The phase structure of the nanocomposite was analyzed using X-ray powder diffraction (XRD) with monochromatized Cu-K α radiation (PANalytical, X'Pert Pro system (Malvern Panalytical, Eindhoven, The Netherlands). The scanning range was set at 10° to 80° with a rate of 0.026, resulting in the acquisition of XRD patterns. A Fourier Transform Infrared (FTIR) Spectrometer (Shimadzu, IRAffinity-1, Kyoto, Japan) within the wavenumber range of (450–4000 cm^{−1}) was used to identify the functional groups, and FTIR spectra were obtained through the KBr pellet method.

Field-Emission Scanning Electron Microscopy (FE-SEM) (Mira3- XMU, TESCAN, Tokyo, Japan) was used to study the morphologies of the samples. The surface topography and roughness of the samples were examined using Atomic Force Microscopy (AFM), with the microscope (JPK BioAFM; Bruker Optics, Berlin, Germany) operated in tapping mode under ambient conditions and analyzed by using the Gwyddion 2.66 open-source software.

2.7. Antibiofilm Test

The experiment to assess *Candida albicans* fungal adhesion was conducted following the methodology outlined by Zupancic et al. [18]. Activation and cultivation of the standard *Candida albicans* (ATCC10231) strain were performed in Sabouraud Dextrose Agar (SDA) for 48 h at a temperature of 37 °C. Following incubation, the individual colonies were collected and suspended in broth, and the turbidity was adjusted to meet the 0.5 McFarland standard. All specimens were sterilized with a Yamato SM-510 steam sterilizer at 121 °C for 15 min and then placed in separate sterile test tubes containing 2 mL of 0.5 McFarland fungus suspension, followed by incubation for 48 h at 37 °C.

The specimens were washed thrice using normal saline in order to detach nonadherent cells. All test samples were transferred into new sterile test tubes in order to loosen the adhered fungal cells, 2 mL of normal saline was added to each tube and the test tubes were placed in a vortex mixer for 3 min [29]. Lastly, a 0.1 mL of the suspension obtained was inoculated on plates of Sabouraud Dextrose Agar and then incubation for 48 h at 37 °C, and the yeast colonies were counted manually. The experiments were repeated thrice to provide the result with accuracy and yielding comparable results. The exact number of colony-forming units per milliliter (CFU/mL) was calculated using the following equation:

$$\text{CFU/mL} = \frac{\text{Number of Colonies} \times \text{Dilution Factor}}{\text{Volume Plated (mL)}} \quad (1)$$

2.8. Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics (version 25.0; IBM Corp., Armonk, NY, USA). To check whether the CFU count data followed a normal distribution, the Shapiro–Wilk test was performed. The results showed that at least one group did not follow a normal distribution ($p \leq 0.05$). Accordingly, non-parametric tests were applied. The Kruskal–Wallis H test was used to compare CFU counts among the five groups (D growth control, D25, D50, D75, and D100), and pairwise comparisons were performed utilizing the Mann–Whitney U test for multiple comparisons. A p -value of <0.05 was considered statistically significant.

3. Results and Discussion

3.1. X-Ray Diffraction Studies (XRD)

The X-ray diffraction technique is a non-destructive technique that is used to examine the morphology of crystallites and phase composition [30]. Figure 3a shows that the pristine

chitosan is amorphous, exhibiting a broad peak around 30° [31]. In contrast, TiO_2 shows sharp diffraction peaks during both the anatase and rutile phases [32].

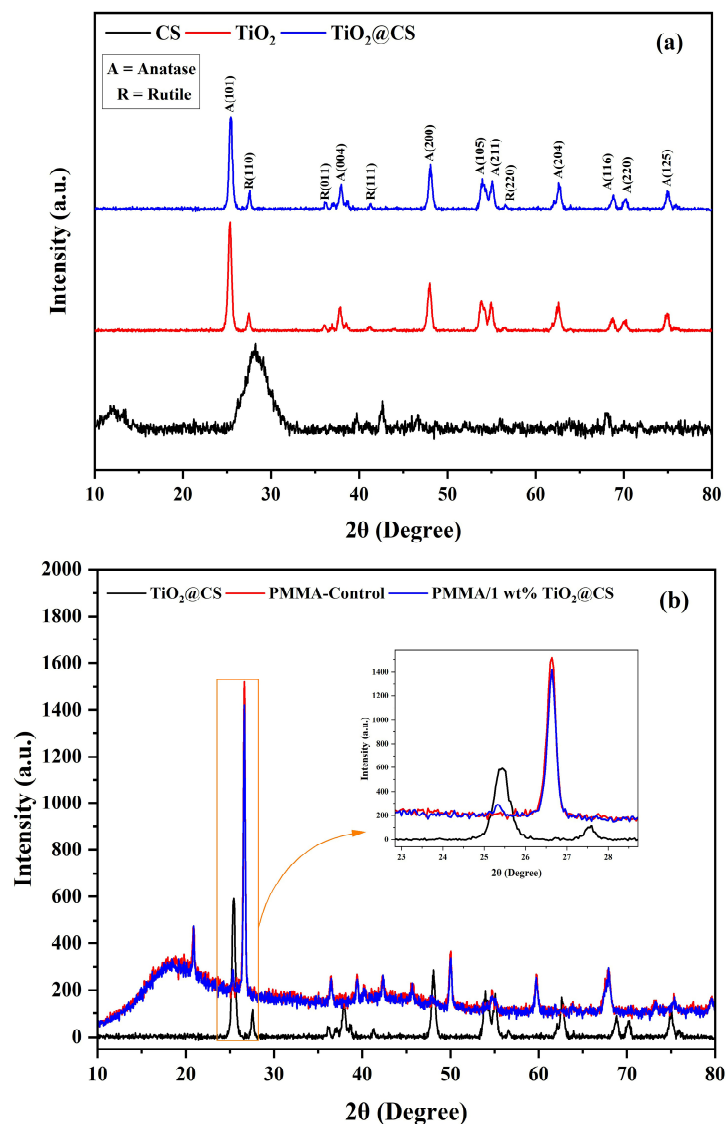


Figure 3. XRD patterns of (a) chitosan (CS), titanium (TiO_2), and titanium-coated chitosan ($\text{TiO}_2@CS$), and of (b) titanium-coated chitosan ($\text{TiO}_2@CS$), PMMA resin, and composite of PMMA/ $\text{TiO}_2@CS$.

After chitosan coating, the $\text{TiO}_2@CS$ composite still exhibits all the characteristics of TiO_2 peaks, which demonstrates that the chitosan coating does not affect the crystallinity or phase composition of the nanoparticles. In the PMMA-based films, as seen in Figure 3b, the PMMA shows crystalline peaks of silica due to a significant ratio of silica within the PMMA resin, but for the 1 wt.% $\text{TiO}_2@CS$ -loaded film, the anatase (101) reflection has lower intensity than expected and is broad because of polymer embedding. This indicates that the TiO_2 crystalline structure is preserved within the polymer matrix [33].

This structural integrity is essential for maintaining photocatalytic activity and antimicrobial function of the composite, supporting its performance in subsequent biological testing.

The X-ray diffraction pattern of PMMA exhibits a broad diffraction halo centered approximately in the range of $2\theta \approx 15\text{--}30^\circ$, which is characteristic of its predominantly amorphous structure. The observed broad peak corresponds to interchain spacing within the PMMA matrix rather than to a true crystallographic plane, confirming the absence of

long-range structural regularity [34]. The presence of this diffuse halo therefore confirms that the resin maintains its amorphous nature without crystalline phase formation.

3.2. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra, as illustrated in Figure 4, show the vibrational features of pure TiO_2 , chitosan (CS), and the chitosan– TiO_2 composite ($\text{TiO}_2@\text{CS}$), confirming effective integration of the biopolymer with the metal oxide. A broad band centered at 3430 cm^{-1} is present in all samples but is notably stronger in CS and $\text{TiO}_2@\text{CS}$, which is attributed to O–H stretching from hydroxyl groups and intermolecular hydrogen bonding within chitosan [35]. The sharp peak at 2922 cm^{-1} is attributed to C–H stretching [36].

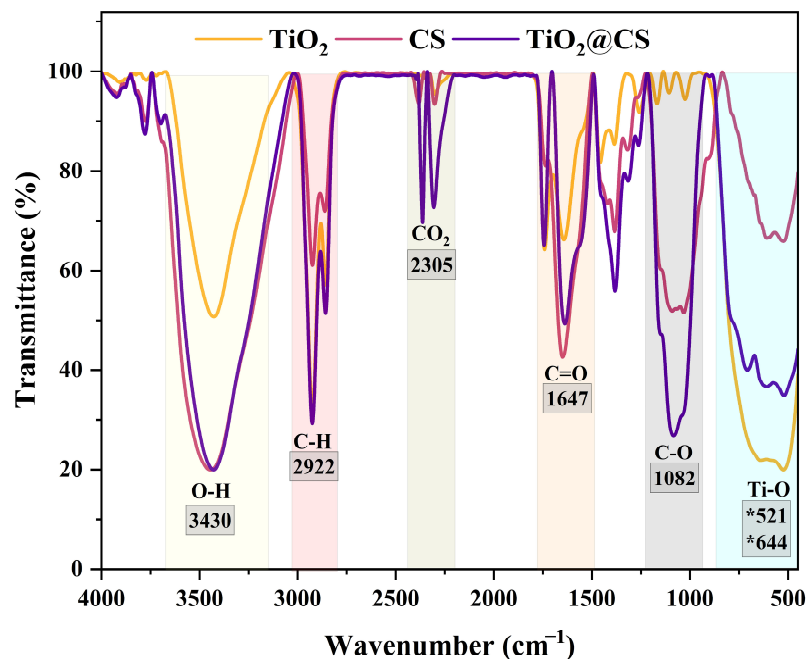


Figure 4. FTIR spectra of titanium oxide nanoparticles (TiO_2), chitosan powder (CS), and chitosan-coated titanium ($\text{TiO}_2@\text{CS}$). * Refers to the fingerprints of Ti–O.

The band around 2305 cm^{-1} is interpreted as asymmetric stretching of CO_2 , likely from ambient air. The sharp peak at 1647 cm^{-1} corresponds to C=O stretching [37]. The band at 1082 cm^{-1} is attributed to C–O vibrations [38]. Additionally, the bands at 521 cm^{-1} and 644 cm^{-1} represent TiO_2 ; they are attributed to Ti–O–Ti lattice vibrations and confirm the integrity of the TiO_2 framework [39]. The observed peak shifts and overlapping bands in the $\text{TiO}_2@\text{CS}$ spectrum corroborate that chitosan successfully coats and interacts with the TiO_2 nanoparticles, confirming the successful synthesis of the core–shell nanoparticle.

Additional absorption features observed in the TiO_2 spectrum can be attributed to surface-related phenomena commonly associated with nanoscale metal oxides. The broad band in the $3200\text{--}3600\text{ cm}^{-1}$ region and the signal near $\sim 1630\text{--}1650\text{ cm}^{-1}$ are assigned to stretching and bending vibrations of surface hydroxyl groups and adsorbed molecular water. Such features are frequently reported for TiO_2 nanoparticles due to their high specific surface area and tendency toward surface hydroxylation under ambient conditions. These hydroxyl groups are known to contribute to interfacial reactivity and hydrogen bonding capacity.

Minor bands appearing in the $1000\text{--}1200\text{ cm}^{-1}$ region may arise from Ti–O–H or Ti–O–C linkages, particularly in hybrid systems where organic components interact with the oxide surface. These interfacial vibrations are more likely to appear in coated or composite materials than in bulk crystalline TiO_2 . Additionally, weak absorption near $\sim 2300\text{ cm}^{-1}$ is

commonly associated with atmospheric CO₂ adsorption during FTIR measurement rather than representing an intrinsic structural vibration of TiO₂.

The FTIR spectra was used to identify the presence of the characteristic chitosan functional groups and their interaction with TiO₂. Such chemical reactions can affect distribution of nanoparticles as well as surface morphology as it is seen in the SEM images. The addition of TiO₂ into the chitosan matrix led to a changed surface topography and enhanced micro and nanoscale heterogeneity, which is in line with the formation of polymer-inorganic hybrid network. These kinds of structural changes have been found to influence microbial adhesion by changing surface energy and roughness and accessibility of antimicrobial functional groups.

3.3. Field Emission Scanning Electron Microscopy (FE-SEM)

The Field Emission Scanning Electron Microscopy (FE-SEM) images presented in Figure 5 provide a comparative morphological analysis of chitosan, TiO₂, and core-shell TiO₂@CS. The porous morphology of pure chitosan, illustrated in Figure 5a, comprises a smooth and continuous polymer matrix with small microvoids. Figure 5b shows TiO₂ nanoparticles that exhibit a dense and agglomerated granular structure composed of roughly spherical particles with a high degree of clustering. In contrast, the core-shell structure illustrated in Figure 5c forming a uniform coating layer of core-shell TiO₂@CS and exhibiting lower agglomeration and better dispersion than pure TiO₂.

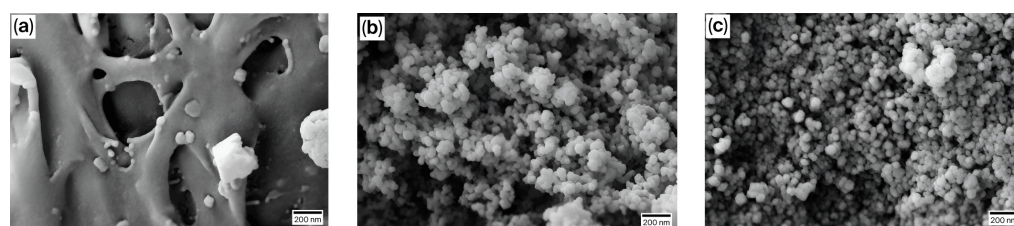


Figure 5. FE-SEM images of (a) chitosan, (b) TiO₂ nanoparticles, and (c) core-shell (TiO₂@Chitosan).

FE-SEM images of the unmodified PMMA resin and the resin modified with TiO₂@Chitosan nanoparticles at different ratios are shown in Figure 6. When TiO₂@Chitosan was embedded with PMMA resin, a smooth, uniform surface was observed at 0.25 wt.%, as seen in Figure 6b, but increasing the ratio to 0.50 wt.%, 0.75 wt.%, and 1 wt.%, as illustrated in Figure 6c–e, led to more pronounced aggregation with larger clusters.

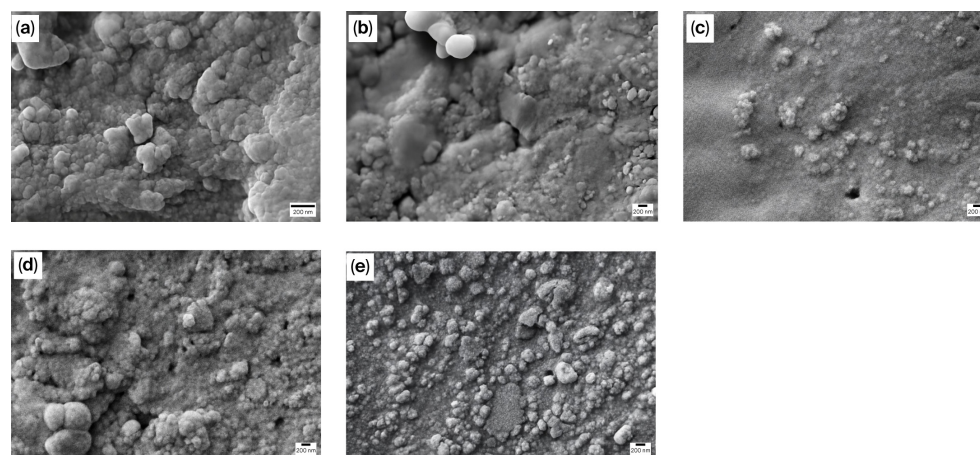


Figure 6. PMMA with different concentrations of the hybrid TiO₂@Chitosan nanoparticle: (a) PMMA resin; (b) 0.25 wt.%, (c) 0.50 wt.%, (d) 0.75 wt.%, and (e) 1 wt.% of PMMA/TiO₂@CS.

The chitosan–TiO₂ (TiO₂@CS) nanoparticle incorporated into the PMMA resin exhibits distinctive morphological features, as shown by the FESEM results. This is consistent with other research showing that biopolymer coatings improve structural stability and reduce the agglomeration of nanoparticles. According to similar studies, TiO₂ nanoparticles were uniformly and successfully dispersed inside a chitosan matrix, producing homogenous, non-agglomerated surfaces with improved antibacterial activity [40,41].

TiO₂@CS nanoparticle is incorporated in the polymer matrix of the PMMA/TiO₂@CS composite, with inconsistent protrusions evident on the surface. This suggests potential for effective load transfer and good interfacial compatibility. As illustrated in PMMA/TiO₂ systems, this behavior is consistent with findings stating that low TiO₂ content in composite films decreases bacterial adherence and emphasizes the danger of nanoparticle aggregation at larger loadings [42].

Furthermore, research on chitosan–TiO₂ membranes indicates that excessive TiO₂ can result in surface aggregation, which can have a detrimental impact on the functional and mechanical properties of the material [30].

In contrast, surface homogeneity is maintained through excellent nanoparticle dispersion, which can be achieved through chitosan coating and appropriate processing. This is essential for both mechanical reinforcement and ensuring consistent antibacterial activity across the composite surface. FESEM confirms that the TiO₂@CS nanoparticle is evenly distributed throughout the PMMA, especially at 0.25 wt.%, as shown in Figure 6b, promoting enhanced structural stability and biofunctional performance.

3.4. Atomic Force Microscopy Analysis (AFM)

The AFM analysis of the composite surfaces reveals distinct modifications in topography across the chitosan (CS), titanium nanoparticles (T), core–shell titanium-coated chitosan (TC), PMMA 3D-printed resin (D), and its TC-blended series (D25–D100), as illustrated in Figures 7a–d and 8a–d. The AFM surface roughness (Ra) values decrease with an increase in the concentration of TC (core–shell titanium-coated chitosan) nanoparticles embedded into the PMMA resin matrix. The pure PMMA resin (D) has the highest Ra value of 2.21 nm, indicating that the PMMA resin surface D is relatively rough [43]. However, the addition of TC nanoparticles at ratios of 0.25 wt.% (D25), 0.50 wt.% (D50), 0.75 wt.% (D75), and 1 wt.% (D100), yields Ra values of 1.7, 1.23, 1.09, and 1.04 nm, respectively, as shown in Table 3. This reduction in roughness is likely due to the uniform distribution and effective surface saturation of the TC nanoparticles, which fill voids and smoothen the surface in the polymer matrix [44]. Augmenting the concentration of nanoparticles may result in the matrix becoming saturated with filler beyond its capacity. The resin must not contain additional filler particles, since this results in material saturation, making the surface rougher and causing structural disruption and adverse effects on its mechanical properties [42].

The moderate intrinsic roughness (0.425 nm) of TC on its own enhances interfacial compatibility with the PMMA arising from the chitosan component and improves the overall interfacial structural density and homogeneity of the nanocomposite surface [45]. In addition, chitosan's biopolymeric nature improves the dispersion stability of metal-based nanoparticles and their incorporation into hydrophobic polymer matrices like PMMA, leading to enhanced surface morphology and decreased roughness [46].

The uniformly distributed TiO₂ in the chitosan matrix contributes to surface roughness texture that develops when the matrix reaches saturation and consequently diminishes the inhibition of biofilm. The effects of surface roughness on adhesion to microorganisms are complicated. Although a very rough surface can offer some space for attachment to microbes, moderate roughness, particularly at a nanoscale, will disrupt adhesion and disrupt the development of biofilms [42].

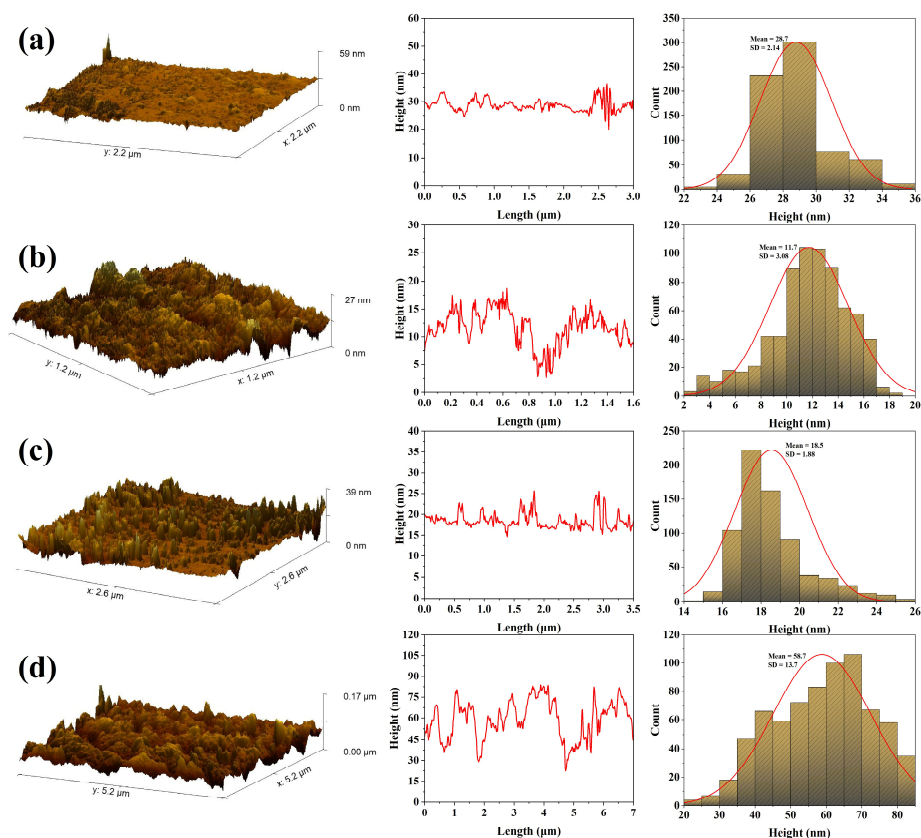


Figure 7. AFM topography of (a) chitosan, (b) TiO_2 , (c) chitosan-coated titanium ($\text{TiO}_2@\text{CS}$), and (d) PMMA.

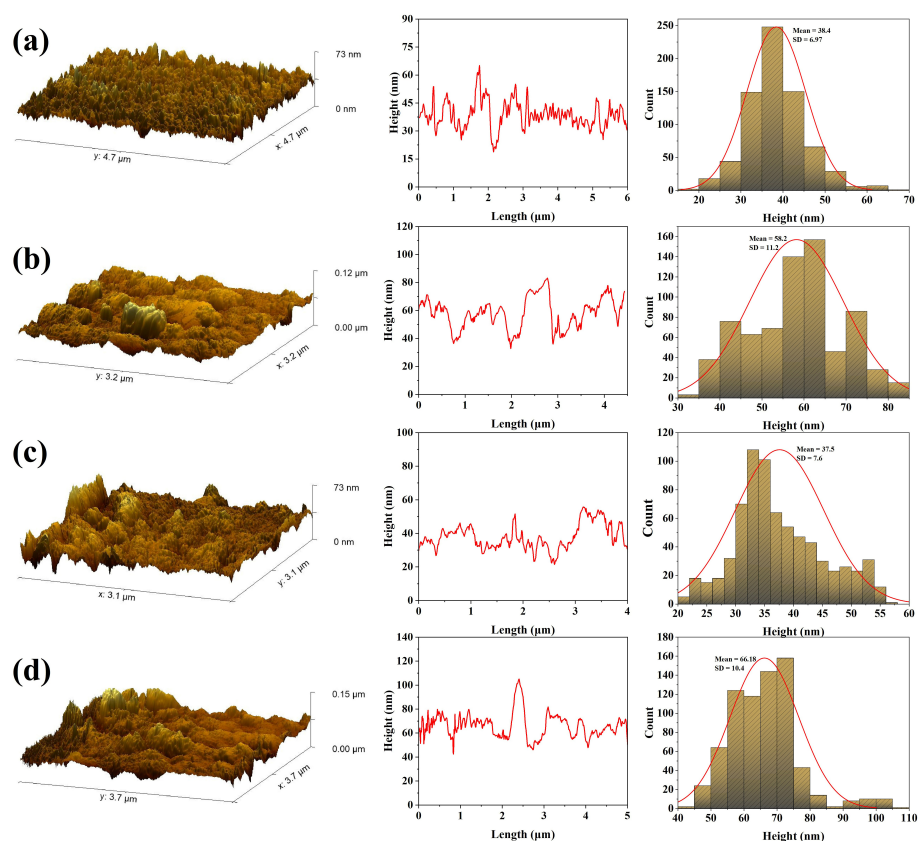


Figure 8. AFM topography of PMMA/ $\text{TiO}_2@\text{CS}$ with ratios of (a) 0.25 wt.%, (b) 0.50 wt.%, (c) 0.75 wt.%, and (d) 1 wt.%.

Table 3. Surface roughness of chitosan (CS), titanium (T), core-shell titanium-coated chitosan (TC), untreated PMMA (D), and TC blended into PMMA at different wt.% ratios.

Parameters	Samples							
	CS	T	TC	D	D25	D50	D75	D100
Ra (nm)	0.288	0.346	0.425	2.21	1.7	1.23	1.09	1.04
Rq (nm)	0.371	0.446	0.555	3.13	2.16	1.63	1.41	1.34
Rt (nm)	3.55	3.11	3.66	29.55	15.77	14.21	10.73	12.42

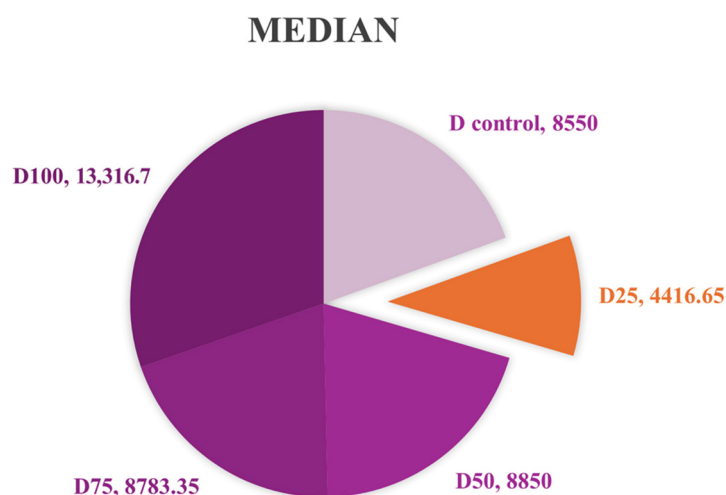
3.5. Antibiofilm Effect

The Kruskal–Wallis test revealed a significant overall difference in biofilm adhesion among the five groups ($H = 14.506$; $df = 4$; $p = 0.006$). Subsequent pairwise Mann–Whitney U tests showed that D25 (0.25%) exhibited a significant reduction in CFU counts compared to the control ($U = 23.0$, $p = 0.043$), indicating that it had reduced biofilm adhesion to the surface of the samples. No significant differences were observed between the control and D50 (0.5%) ($p = 0.739$) or D75 (0.75%) ($p = 0.796$). In contrast, D100 (1%) demonstrated a significant increase in CFU relative to the control ($U = 21.0$, $p = 0.029$), suggesting that it had greater biofilm adhesion to the surface, as shown in Table 4 and Figure 9.

Table 4. Median, IQR (Interquartile Range), p -values, and significance (* $p < 0.05$) of the groups compared to the control group.

Kruskal–Wallis $H = 14.506$ p -Value = 0.006 *						
Group	N	Minimum	Maximum	Median	IQR	p -Value
D control (0%)	10	4266.70	17,000.00	8550.00	9208.35	
D25 (0.25%)	10	2533.30	17,533.30	4416.65	4191.67	0.043 *
D50 (0.5%)	10	3133.30	16,866.70	8850.00	6758.35	0.739
D75 (0.75%)	10	2800.00	16,733.30	8783.35	8233.35	0.796
D100 (1%)	10	7600.00	25,466.70	13,316.70	6666.65	0.029 *

* p -values represent Mann–Whitney U tests pairwise comparisons versus control.

**Figure 9.** Comparison of the median CFU counts of the PMMA/TiO₂@CS nanocomposite at different concentrations and the D control (0%).

These results affirm that *C. albicans* biofilm growth on the resin sample surface was best inhibited by D25 (0.25%) treatment. This confirms earlier research findings, which

indicate that lower concentrations of TiO₂ and chitosan exhibit antimicrobial properties and possess biocompatibility and sufficient strength [10,47].

The efficacy of titanium dioxide nanoparticles as anti-microbial agents depends on a series of parameters such as their size, morphology, shape, photocatalytic activities and crystal structure [10,48,49]. TiO₂ affects microbial cells by disrupting their cell wall and membrane, causing DNA damage and interfering with both DNA replication and protein synthesis [50]. Compared to the unmodified resin, TiO₂ nanoparticles demonstrate notably antifungal activity against *C. albicans* when incorporated into 3D-printed denture base resin at concentrations of up to 0.50 wt.%, without exhibiting toxicity toward human gingival fibroblasts [11,22].

The biological properties of chitosan will range; chitosan exhibits antimicrobial activity that is strongly dependent on environmental pH. Under acidic or mildly acidic conditions, protonation of its amino groups (NH₃⁺) enables electrostatic interactions with negatively charged molecules of fungal cells [51]. Chitosan was shown to have great potential as an anti-candida agent since it was active against both the planktonic and sessile states of *C. albicans* and displayed notably important activity during different stages of biofilm succession-adhesion, formation, maturation and co-aggregation [52,53].

The better antibiofilm efficacy of the 0.25 wt.% composite is due to synergetic effect of nanoparticle dispersion, surface topography and surface chemistry. At this concentration, the uniform core-shell structure of the chitosan-TiO₂ nanoparticles provides moderate levels of nanoscale roughness and high inclusion of exposed reactive surfaces to facilitate effective contact with microbial cells. Increased availability of protonated amino groups afforded by chitosan favors electrostatic attraction to the negatively charged fungal cell walls to disrupt the cell membrane. At the same time, the remaining TiO₂ surface activity promotes reactive interactions that further disrupt cell integrity. The core-shell nanocomposite architecture, combined with the effects of chitosan functional groups confirmed by FTIR and the altered surface morphology revealed by SEM, which influence microbial attachment and viability together, possibly enhances these effects by increasing surface contact area, stabilizing nanoparticle dispersion, and creating a multifunctional interface that interferes with microbial adhesion and early biofilm development. This structural integration explains the observed reduction in *Candida albicans* adhesion and biofilm formation on modified acrylic surfaces. In contrast, increased nanoparticle loadings encouraged agglomeration that led to decreased effective surface area and uneven surfaces facilitating microbial adhesion and compromising anti-microbial activity.

Consequently, the antibiofilm property of the core-shell chitosan-TiO₂ nanoparticle is attributed to the synergic effect of both nanoparticles. The mechanism action of the CS-TiO₂ nanoparticle is suggested to be different, Longo et al. [54] explained that TiO₂ could be present or trapped on the nanocomposite surface, thus enhancing their antimicrobial activity due to the generation of electron-hole pairs without the need of light-induced radiation. Similar results were reported Kamal et al. [55] suggested that the antibacterial mechanism of the hybrid composites could be associated with the interaction of electrostatic charges between CS-TiO₂ (positive) and bactericidal membranes (negative), which may promote a membrane cell alteration, blocking nutrient intake and affecting the viability and normal cell growth.

Shi et al. [56] demonstrated that gauzes infused with a CS-TiO₂ emulsion effectively inhibited the proliferation of *E. coli* (99.9%), *A. niger* (100%), and *C. albicans* (78%); moreover, they can be reused up to eight times without diminishing their antimicrobial efficacy. While Shi et al. found 78% inhibition using CS-TiO₂ coatings, significant reduction in the present study was only observed at 0.25 wt.%, indicating that a lower loading in 3D-printed PMMA systems may be due to the interaction of the polymer with the nanoparticles.

Similarly, Xiao et al. [57] demonstrated that a CS–Fe–TiO₂ composite coating showed long-term stability, maintaining its antifungal (*C. albicans* and *A. niger*) and antibacterial (*E. coli*) properties for up to a year in storage. This means that the coating has a long shelf life, and its antibacterial properties are unlikely to degrade over time, which makes it a good choice for long-term biomedical use. Anaya-Esparza et al. [17] also demonstrated that a CS–TiO₂ composite exhibited exceptional antimicrobial activity against yeast, Gram-positive and Gram-negative bacteria, and molds.

The reduced aggregation observed at 0.25 wt.% correlates with the highest antibiofilm performance, supporting the hypothesis that nanoparticle dispersion directly affects microbial adhesion.

Interestingly, the D100 group (1%) not only failed to inhibit biofilm formation but also caused a significant increase in CFU counts compared to the control, meaning that the growth and attachment of *C. albicans* were much stronger. This unexpected finding can be attributed to nanoparticle aggregation as seen in the FESEM images, which may lead to surface irregularities and reduce the effective surface area available for microbial interaction or non-uniformity of the polymers within the matrix, and may result in surface flaws that promote microbial adherence, thereby reducing antimicrobial effectiveness [22,58,59].

From a clinical perspective, addition of 0.25 wt.% core–shell chitosan–TiO₂ into 3D-printed denture base resin is a possible solution to the denture-related *Candida* infections, including denture stomatitis. As the 3D-printed prostheses gain more popularity and the absence of microbial resistance is recognized as a shortcoming, this solution can be applied as some sort of preventive optimization without one having to make serious adjustments to the process of producing them. Moreover, employing a biocompatible nanocomposite aligns with the current focus in dentistry on multifunctional and safer dental materials.

Although the present study shows promising results of incorporating 0.25 wt.% core–shell chitosan–TiO₂ nanoparticles in 3D-printed denture base resin, there are some limitations that need to be acknowledged. First, the investigation was performed under in vitro static conditions, which did not completely mirror the oral environment with its complex biology (saliva flow, temperature fluctuation, masticatory forces, microbial diversity).

The biofilm model used was limited to *Candida albicans*, while denture-associated infections are multispecies associated infections with bacterial–fungal interactions that may modify the adhesion behavior and resistance. In addition, the study did not evaluate long-term mechanical properties or the effects of aging, such as water sorption, thermal cycling or wear, which are important in terms of the durability in the clinic. The extrapolation of published work to cytotoxicity and biocompatibility was done instead of the actual test, and thus, restricts the drawbacks that can be made on the topic of safety. Lastly, aggregation appeared with an increase in nanoparticles loadings; hence, there is a possibility that the fabrication method should be improved. Such restrictions imply that the limitations of the study will need more in vivo and long-term studies before the clinical use can be entirely justified.

Further studies on the long-term biocompatibility of nanocomposites, maintenance of mechanical properties and their performance on multispecies biofilms in dynamic oral contexts should be undertaken further. In addition, exploration of synergistic associations with other antibacterial agents can also be useful in enhancing the protective characteristics of denture base materials.

4. Conclusions

The addition of a core–shell chitosan–TiO₂ nanoparticle to 3D-printed denture base resins demonstrated a significant antibiofilm effect against *C. albicans* at a concentration of 0.25 wt.%. This was probably as a result of an equal distribution of the nanoparticles at this

concentration and the roughness of the resin which promotes lower microbial adhesion. Conversely, higher concentrations could have led to a lesser effect because nanoparticles could cluster and aggregate, which would disrupt the uniformity of the surface, thus reducing antibiofilm efficacy. Under the conditions of this study, these findings suggest a potential role for such modifications in limiting early-stage fungal colonization; however, further investigations using multispecies biofilm models are required to fully elucidate their effectiveness against clinically relevant denture-associated biofilms.

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Abbreviations

The following abbreviations are used in this manuscript:

CS	Chitosan
TiO ₂	Titanium Dioxide
3D	Three Dimensional
PMMA	Polymethyl Methacrylate
XRD	X-Ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
FESEM	Field Emission Scanning Electron Microscopy
AFM	Atomic Force Microscopy
<i>C. albicans</i>	<i>Candida albicans</i>
CFU	Colony Forming Unit
CAD-CAM	Computer-Aided Design/Computer-Aided Manufacturing
TiO ₂ @CS	Core–Shell Chitosan–Titanium Dioxide Nanocomposite
TEM	Transmission Electron Microscopy
NH ₄ OH	Ammonium Hydroxide
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
NPs	Nanoparticles
ISO	International Organization for Standardization
STL	Standard Tessellation Language
UV	Ultraviolet Radiation
FEPA	Federation Of European Producers of Abrasives
ATCC10231	American Type Culture Collection for <i>Candida Albicans</i> 10231

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