Original Article

Antimicrobial Effect of Three Different Nanoparticles-Modified 3D-Printed Denture Resin: An *In Vitro* Study

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ABSTRACT

Aim: This study aimed to determine the antibacterial effectiveness of adding zirconia nanoparticles (NPs; ZrO_2NPs), silver NPs (AgNPs), and titanium dioxide NPs (TiO_2NPs) in various concentrations to three-dimensional (3D)-printed denture resin against *Candida Albicans*, *Streptococcus pyogenes*, and *Staphylococcus aureus*, this study was carried out. **Materials and Methods:** The antimicrobial efficacy of 150 disk-shaped specimens with a diameter of 15 mm × 2 mm of unmodified (n = 15) and modified (n = 135) 3D-printed denture resin specimens after the addition of silanated ZrO_2NPs, AgNPs, or TiO_2NPs (n = 45) in varying concentrations (n = 15) of 0.5%, 1%, and 1.5% were compared using three oral bacteria (*S.pyogenes*, *S. aureus*, and *C. albicans*) as test subjects. Antimicrobial activity was tested by disk diffusion methods. **Results:** According to the results, when the three tested NPs (ZrO_2NPs, AgNPs, and TiO_2NPs) were added, the bacterial count significantly decreased compared with the unmodified 3D-printed resin. Additionally, the findings showed that as the concentration of the studied NPs increased, so did their antibacterial activity. At 1.5% and 1% concentrations, the AgNPs' antibacterial activity was negligible. However, the *in vitro* study's findings showed that, in terms of the kinds of microorganisms studied, there were no appreciable variations between the three tested NPs. **Conclusion:** The inclusion of ZrO_2NPs, AgNPs, and TiO_2NPs significantly had antimicrobial action against (*S. pyogenes*, *S. aureus*, and *C. albicans*).

Keywords: *Antibacterial activity, denture resin, nanoparticles, 3D-printed*

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Introduction

Comparing computer-aided manufacturing (CAM) to conventional fabrication methods demonstrates that it has the benefit of avoiding several error-prone stages, such as impression, waxing, and casting.[1,2] This is supposed to increase prosthesis precision and reduce error-causing causes. Additionally, because modeling and production are automated processes, there is a general reduction in fabrication time and cost.[3] The technology of computer-aided design (CAD)–CAM, which comprises subtractive methods and additive methods, popularly known as three-dimensional (3D) printing and fast prototyping, can currently be used to create removable dentures.[4]

A manufacturing process that constructs objects one layer at a time is known as 3D printing.[4-6] The capacity

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of 3D-printing technologies to immediately receive CAD data and swiftly create a new digital model has enabled the revolution of 3D printing in dentistry, which makes it possible to fabricate a full denture foundation without the use of molds or cutting tools.[4,7] It has advantages in terms of the enhancement of tissue adaptation and the simplicity of duplicating preexisting dentures in addition to the shorter time that operations and lab work required.[6-9]

The use of 3D printing technology may produce dental prostheses with undercuts, voids, intricate internal geometrical components, and anatomical landmarks while also eliminating operator and procedural errors.[5] The 3D-printed resin was used as denture base resin because of its quick development as well as due to the numerous benefits it offers over traditional approaches. Moreover, the next industrial revolution's key technology is another way to define it.[4]

A serious issue that became apparent over time with repeated use of acrylic resin material is its insufficient antibacterial activity, which permits bacterial and fungal species to cling and colonize the surface of the restoration.[10] To prevent bacterial growth around the restoration, antibacterial restorative material would be ideal.[11] The introduction of nanoparticles (NPs) into dentistry was done to improve the material qualities.[5] However, NP-based compounds have been used as a unique weapon against microbial resistance and multi-drug resistance.[12] NPs offer higher levels of fungicidal action than conventional antifungal drugs because they can enter host cells and tissues more successfully, even in small amounts.[13]

This study was conducted to evaluate the antimicrobial activity of incorporating zirconia NPs (ZrO_2NPs), silver NPs (AgNPs), or titanium dioxide NPs (TiO_2NPs) with different concentrations to 3D-printed acrylic denture base resins against (*Staphylococcus aureus*, *Streptococcus pyogenes*, and *Candida Albicans*).

Materials and Methods

This randomized study was conducted on nine groups of resin that had been modified (n = 135) using silanated ZrO_2NPs, AgNPs, or TiO_2NPs (Sigma–Aldrich, Burlington, MA, USA) with an average granular size of 40 nm and a surface area of 9 m^2/g based on analyses by transmission electron microscope and scanning electron microscope in different concentrations of 0.5%, 1%, and 1.5%.

This study was done after approval from the ethical committee Faculty of Dental Medicine, Al-Azhar University, Assiut, Egypt (Approval code: AUAREC20210003-12). Informed written consent was obtained from all patients.

Specimens' preparation

To improve the bonding between the individual NPs and the resin matrix the various NPs were added after the silane coupling agent had been dissolved in acetone, and the mixture was agitated for 60 min, and the acetone was then removed using a rotary evaporator, and the silanized NPs were obtained after cooling. The silanized NPs were introduced to the 3D-printing resin after being weighed on an electronic scale and the fluid resins containing silanized NPs were fully mixed and stirred for 30 min using a magnetic stirrer.[5] A disc shape specimen with a diameter of (15 mm × 2 mm) was virtually created using open-source CAD technology and CAD software (3Shape CAMbridge) and saved in an STL file formatted. The previously created disc design was uploaded as an STL file to the software, which was then received by a 3D printer (EPAX 3D, Morrisville, NC, USA) utilizing digital light printing. The photopolymerized liquid methyl methacrylate monomer was poured through the nozzle of the 3D printer in successive layers with a 50 μm layer thickness at 90°.[14] Isopropyl alcohol solution (99.9%; ultra-pure; Sigma–Aldrich) in an ultrasonic cleaner (Fisherbrand™; Thermo Fisher Scientific, Waltham, MA, USA) was used to clean and remove any residues from the printed discs before curing. The specimens were then polymerized by ultraviolet (UV) radiation using a specialized UV light-curing box (Bredent, Bre. Lux Power Unit 2, Senden, Germany) under nitrogen pressure. The post-curing procedure involved wiping each specimen with isopropyl alcohol and submerging it in a bowl of glycerol. According to the manufacturer's instructions, the post-curing procedure took place in a post-curing unit for 10 min[5,15] [Figure 1].

Antimicrobial testing procedures

Each specimen was sterilized with 70% isopropyl alcohol before testing its antimicrobial activity. To create the adjusted inoculum, a reference strain of *C. albicans* (ATCC 10231) was grown on Sabouraud dextrose agar at 30°C for 48 h.[5,11] The strain of *S. pyogenes* (UA159) was employed, and the bacteria were grown in 5 mL of brain–heart infusion broth culture for 24 h in an aerobic environment at 37°C.[11] However, to create the adjusted inoculum, Luria agar medium was utilized for *S. aureus*.[16] Each group specimens were sterilized and then placed on a 48-well plate with 500 mL of each media and 50 mL of inoculated bacterial suspension for 24 h.[5,11] The bacterial solution was adjusted to 1.0×10^6 colony-forming units (CFUs)/mL for standardization

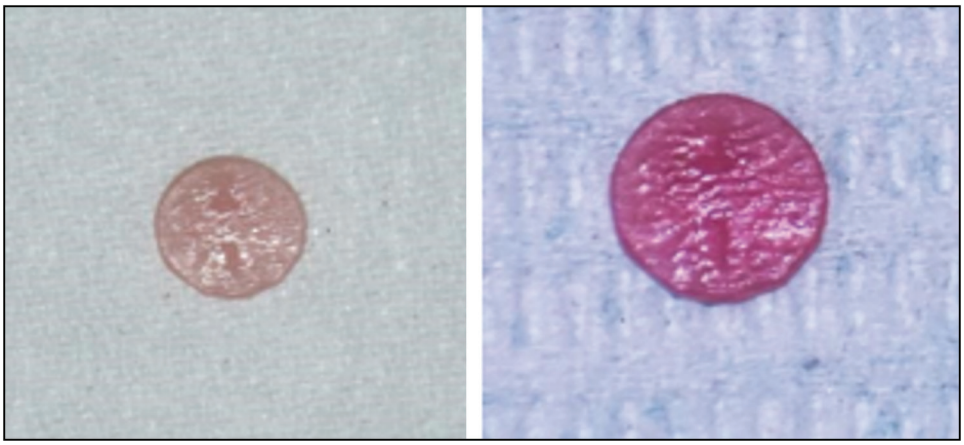

Figure 1: A photograph showing three-dimensional-printed specimens

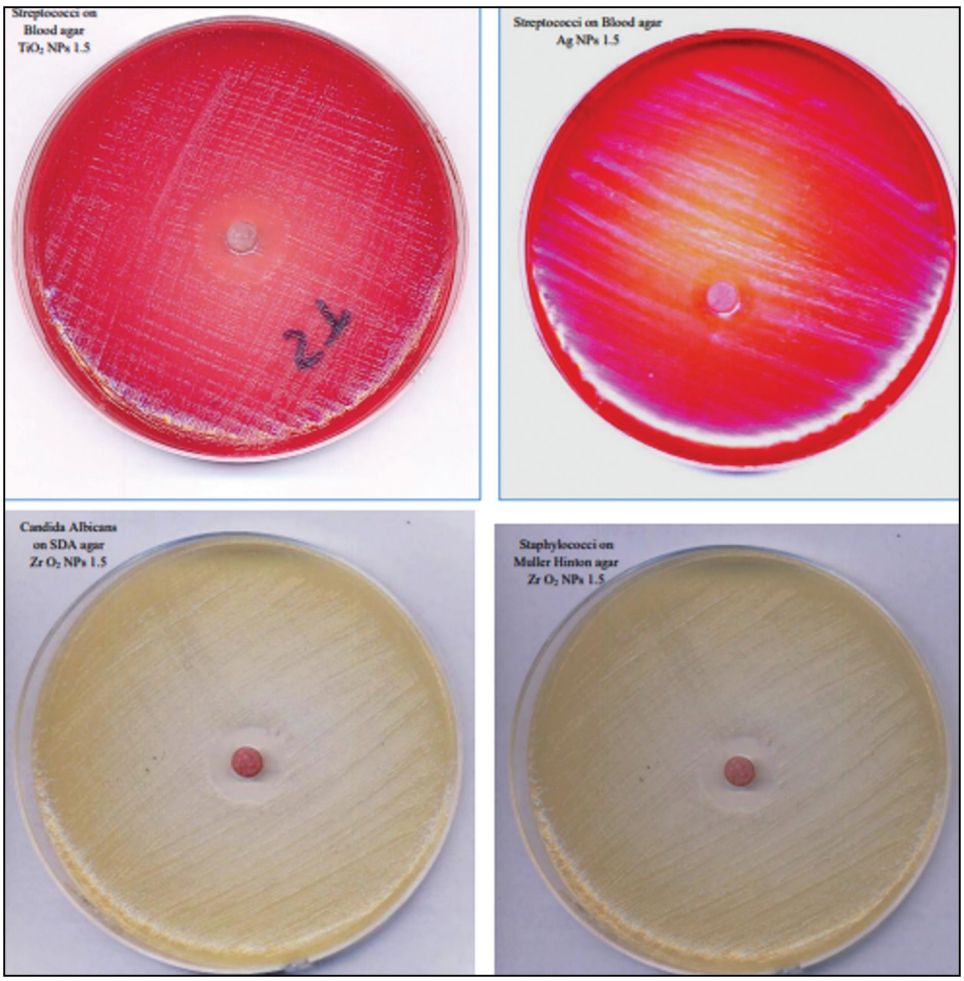

Figure 2: A photograph of agar media with three-dimensional-printed specimens

of the bacterial count.[11] Pipette-harvested bacteria from biofilms on discs were diluted to a concentration of 10^6 times before being planted into agar plates in the amount of 50 mL.[5,11] The bacterial cells were counted using a CFU approach that involved streaking a properly diluted solution over agar media and incubation for 24 h at 37°C.[5] Three times the trials were run with the same outcomes in duplicates [Figure 2].

Sample size

Power analysis was used to count the *in vitro* samples. Using World Health Organization calculations, a

Table 1: Comparison of the antibacterial effect at various concentrations

	0.5% concentration				
	Control (CFU)	ZiO_2NPs (CFU)	AgNPs (CFU)	TiO_2NPs (CFU)	*P* value
S. aureus	1.0 ± 0.00 × 106 Aa 95% CI (1:1)	734 ± 65.8 × 103 Ba 95% CI (697.56:770.44)	740 ± 66.9 × 103 Ba 95% CI (702.95:777.05)	721 ± 69.7 × 103 Ba 95% CI (682.4:759.6)	0.002*
S. pyogenes	1.0 ± 0.00 × 106 Aa 95% CI (1:1)	726 ± 66.5 × 103 Bb 95% CI 689.17:762.83)	731 ± 66.8 × 103 Bb 95% CI (694:768)	720 ± 69.9 × 103 Bb 95% CI (681.29:758.71)	0.0015*
C. albicans	1.0 ± 0.00 × 106 Aa 95% CI (1:1)	763 ± 63.9 × 103 Bc 95% CI (702.95:777.05)	774 ± 49.9 × 103 Bb 95% CI (746.36:801.64)	756 ± 82.5 × 103 Bc 95% CI (710.31:801.69)	0.008*
P value	1 ns	<0.001*	<0.001*	<0.001*	
	1% concentration				
	Control (CFU)	ZiO_2NPs (CFU)	AgNPs (CFU)	TiO_2NP (CFU)	*P*-value
S. aureus	1.0 ± 0.00 × $10^{6\,Aa}$ 95% CI (1:1)	394 ± 47.7 × $10^{2\,Ba}$ 95% CI (367.58:420.42)	396 ± 36.4 × $10^{2\,Ba}$ 95% CI (375.58:416.16)	322 ± 48.2 × $10^{2\,Ca}$ 95% CI (295.31:348.69)	<0.001*
S. pyogenes	1.0 ± 0.00 × $10^{6\,Aa}$ 95% CI (1:1)	383 ± 50.1 × $10^{2\,Bb}$ 95% CI (355.25:410.75)	390 ± 38.1 × $10^{2\,Bb}$ 95% CI (368.9:411.1)	310 ± 47.0 × $10^{2\,Cb}$ 95% CI (283.97:336.03)	<0.001*
C. albicans	1.0 ± 0.00 × $10^{6\,Aa}$ 95% CI (1:1)	400 ± 41.8 × 10^{2} bc 95% CI (376.85:423.15)	418 ± 43.1 × $10^{2\,Bb}$ 95% CI (394.13:441.87)	324 ± 47.2 × $10^{2\,Cc}$ 95% CI (297.86:350.14)	<0.001*
P value	1 ns	<0.001*	<0.001*	<0.001*	
	1.5% concentration				
	Control (CFU)	ZiO_2NPs (CFU)	AgNPs (CFU)	TiO_2NP (CFU)	*P*-value
S. aureus	1.0 ± 0.00 × $10^{6\,Aa}$ 95% CI (1:1)	268 ± 34.7 × 10^{2Ba} 95% CI (248.78:287.22)	351 ± 17.8 × $10^{2\,Ca}$ 95% CI (341.14:360.86)	229 ± 6.5 × $10^{2\,Da}$ 95% CI (225.4:232.6)	<0.001*
S. pyogenes	1.0 ± 0.00 × $10^{6\,Aa}$ 95% CI (1:1)	263 ± 37.8 × $10^{2\,Bb}$ 95% CI (242.06:283.94)	345 ± 16.9 × $10^{2\,Cb}$ 95% CI (335.64:354.36)	223 ± 5.7 × $10^{2\,Db}$ 95% CI (219.84:226.16)	<0.001*
C. albicans	1.0 ± 0.00 × $10^{6\,Aa}$ 95% CI (1:1)	279 ± 38.9 × 10^{2} bc 95% CI (335.64:354.36)	370 ± 18.0 × $10^{2\,Cb}$ 95% CI (360.03:379.97)	227 ± 17.5 × $10^{2\,Dc}$ 95% CI (217.31:236.69)	0*
P value	1 ns	<0.001*	<0.001*	<0.001*	

ns = nonsignificant, CFU = colony forming units, CI = confidence interval, ZiO_2NPs = zirconia nanoparticles, AgNPs = silver nanoparticles, TiO_2NPs = titanium dioxide nanoparticles, *S. aureus* = *Staphylococcus aureus, S. pyogenes* = *Streptococcus pyogenes, C. albicans* = *Candida albicans.*

Different uppercase letters mean significant in the same row.

Different lowercase letters mean significant in the same column.

*Significant ($P < 0.05$).

research power of 80%, a significance level of 5%, and an error margin of 5% were determined. For 150 specimens in total, 10 groups (n = 15/group) were made, one group of 3D-printed resin that was left unmodified, and nine groups of resin that had been modified (n = 135).

Statistical analysis

Statistical analysis was done by Statistical Package for Social Sciences version 26 (IBM Inc., Chicago, IL, USA). Quantitative variables were presented as mean and standard deviation and compared between the three groups utilizing the analysis of variance (ANOVA; *F*) test. A two-tailed *P* value of <0.05 was considered statistically significant.

Results

The normality results showed that the data originated from a normal distribution (parametric data) in all groups ($P > 0.05$). The ANOVA results revealed that the 0.5%, 1%, and 1.5% modified ZiO_2NPs, AgNPs, and TiO_2NP 3D-printed resin groups had significantly higher antimicrobial activity than in the unmodified 3D-printed resin (control group). However, for intergroup comparison, the Tukey honestly significant difference (HSD) test results revealed that there was no statistically significant difference between the 0.5% modified ZiO_2NPs, AgNPs, and TiO_2NP 3D-printed resins groups regarding *S. aureus*, *S. pyogenes*, and *C. albicans* microorganisms at 0.5% NPs concentrations. Furthermore, in intergroup comparison, there was no statistically significant difference between 1% modified ZiO_2NPs and AgNPs 3D-printed resin groups regarding *S. aureus*, *S. pyogenes*, and *C. albicans* microorganisms. However, there was a statistically significant difference in the antimicrobial activity between the 1.5% modified ZiO_2NPs, AgNPs, and TiO_2NP 3D-printed resin groups [Table 1].

The ANOVA results also revealed that the antimicrobial activity significantly increased with the increase of

ZiO_2NPs, AgNPs, and TiO_2NP, concentration from 0.5% to 1.5% in the 3D-printed resin. For intergroup comparison, the Tukey HSD test results revealed that there was a statistically significant difference between the antimicrobial activity of the three tested concentrations (0.5%, 1%, and 1.5%) of the modified ZiO_2NPs and TiO_2NPs 3D-printed resin groups for all tested microorganisms. However, there was a statistically significant difference between the antimicrobial activity of the 0.5% and 1% tested concentrations; however, there was no significant difference between the antimicrobial activity of the 1% and 1.5% tested concentrations of the modified AgNPs 3D-printed resin groups for all tested microorganisms. [Table 1]

Discussion

The growth of infection may be facilitated by adhesion to surfaces made of acrylic resin. In the end, this could result in different degrees of denture-induced stomatitis, which affects 70% of people who wear dentures.[5] Therefore, in this current experiment, three different types of microorganisms (*S. aureus*, *S. pyogenes*, and *C. albicans*) were selected to be tested. This is because it was stated that *C. albicans* is a common bacterium that can cause *Candida*-associated denture stomatitis.[11] Therefore, it was incorporated into the current investigation. Furthermore, *S. pyogenes*, which is frequently found on the surface of acrylic resins, can compete for bidding sites but may also encourage yeast adherence.[13,17,18]

The introduction of NPs into dentistry was done to improve the material qualities.[5] For material used in 3D printing, NPs might make an appropriate reinforcing approach.[4] To create antibacterial composites, three inorganic antibacterial NPs (ZrO_2NPs, AgNPs, and TiO_2NPs) were chosen in this experiment to incorporate into the 3D-printed resin because of their established antibacterial activities, in particular, have drawn significant attention among the many NPs that have been utilized.[11,19-23]

In this current investigation NPs concentrations of 0.5%, 1%, and 1.5% were selected because NPs have been studied in the past for their impacts on the characteristics of 3D-printed denture bases, including impact strength, elastic modulus, hardness, and surface roughness.[4,5] In this current investigation, CFU was used because it is one of the methods most frequently used for CFUs that is, easy to use, inexpensive, and can calculate the degree of growth inhibition.[5]

The results revealed that all three tested NPs-modified 3D-printed resins inhibited the *S. aureus*, *S. pyogenes*, and *C. albicans* microorganisms significantly when compared with the unmodified 3D-printed resin at all concentrations. The mechanism involves the production of active oxygen species, which inhibit the normal budding process of pore formation in the cell wall. This causes the cell wall to disintegrate via ion outflow, which inhibits fungal growth by disturbing cell function.[24]

These results agreed with the results of the previous studies by Chen *et al*.[11] and Khattar *et al*.[5] stated that the polymethyl methacrylate (PMMA) resin or photopolymerizes 3D-printed resin had no antimicrobial effect against different tested bacteria. Altarazi *et al*.[25] found that the concentrations of 0.10, 0.25, and 0.50 wt.% of TiO_2NPs caused a significant reduction in the number of *Candida* cells attached to the surface of the specimens. Moreover,[26] demonstrated that the addition of TiO_2NPs with 1% and 3% concentrations to PMMA has a greater antimicrobial effect with a reduction of the colonization of *S. aureus*, *Staphylococcus epidermidis*, and *C. albicans* than the conventional dentures.[27] showed a significant increase in antifungal activity of the 3D-printed denture base resin after adding ZrO2NPs.

According to the results of this study, adding ZrO_2NPs, AgNPs, and TiO_2NPs to the resin used in 3D printing caused a decrease in the number of tested bacterial microorganisms. This might be a result of NPs' antimicrobial action, which has been documented in multiple investigations.[5,16,28,29] The decrease in the number of bacteria could be related to the presence of NPs on the specimen surface near the bacterial cell membrane, which could give the modified resin antifungal properties.[5,11]

Through a variety of methods, including hydrophobic contact, electrostatic attraction, and van der Waals forces, NPs can meet one another as they travel through the bacterial cell membrane and disrupt the metabolic process. This interferes with the normal budding process and changes the form and functionality of the cell membrane.[5,10] This also could explain the significant antimicrobial results of all tested NPs against the three different microorganisms in this experiment.

Additionally, the microbial cell's disintegration via the development of holes, which results in ion outflow and structural alterations to the cell that ultimately result in cell death, may have been the origin of the antibacterial effect.[16,30] Moreover, reactive oxygen species that may be produced by NPs and cell membranes interact to enhance cell permeability, which leads to the release of intracellular contents and eventually to cell death.[5,16,28,29]

The results of this present study revealed that there was a significant increase in antimicrobial activity of the modified 3D-printed resins with an increase in different NP concentrations from 0.5% to 1.5%. This may be due to the increased density of ZrO_2NPs, AgNPs, and TiO_2NPs on the specimens' surfaces, it was hypothesized that high concentrations would have a higher impact based on the discovery of antibiofilm actions at low concentrations.[5,29,31]

However, the results of this study showed that there was an insignificant increase in the antimicrobial activity of different AgNPs when concentration increased from 1.0% to 1.5%. This could be related to the agglomeration of these NPs and cluster formation within the resin matrix or at the resin surface may be responsible for the correlation between greater concentrations (1.5%) and increased cell proliferation when compared with low concentrations (0.5% and 1.0%).[5,29] On the other hand, in line with prior work,[5,30] the CFU results revealed a negligible reduction in the bacterial count with the addition of NPs in higher concentrations.

The results of this present study revealed that the AgNPs had a significantly lower antimicrobial effect than the ZiO_2NP and TiO_2NP at 1.5% concentration. These results could be attributed to the higher tendency of AgNPs to cluster as it was found that AgNPs less than 200 nm in diameter tend to spontaneously combine, and their stability in air, water, or sunlight is insufficient for long-term use.[11] It was stated that in comparison to isolated NPs, clustered NPs have a smaller surface area when they aggregate. Only the surfaces near the cluster edges showed antifungal activity, with the cluster's core being obscured by the NP agglomeration.[5,32]

Moreover, the results of this study revealed that the ZiO_2NPs and AgNPs had a lower antimicrobial effect than TiO_2NPs at concentrations of 1% and 1.5%. It was affirmed that when the 3D-printed plastic is photopolymerized by UV radiation due to the photocatalytic properties of these TiO_2NPs, their antibacterial capabilities can be further increased by UV illumination. To achieve more efficacy with less concentration, 3D printing was preferred.[33] Moreover, this may be a result of TiO_2's inherent photocatalytic properties to UV irradiation that is, used to polymerize the 3D-printed resin. Another restriction on the use of TiO_2 is the quick recombination of photogenerated electron–hole pairs.[11] Since it was identified that UV in 3D printing activates TiO_2's crystalline form and produces electrons, ROS, superoxide, and OH•, it may be to blame for this effect of the TiO_2.[28]

The results of this present study revealed that there was no statistically significant difference in the antimicrobial activity of each of the ZrO_2NPs, AgNPs, or TiO_2NPs against the different types of tested microorganisms. Although the precise mechanism of action is still unclear, the antibacterial mechanism of NPs can be loosely separated into three groups. The following is a description of the antibacterial mechanisms: interacting with bacterial proteins and interfering with protein synthesis; engaging with bacterial (cytoplasmic) deoxyribonucleic acid (DNA) and blocking DNA replication; interacting with the peptidoglycan cell wall and membrane and causing cell lysis.[34] Because the environment in the oral cavity is dynamic, this study's specimens were examined under circumstances that were different from those in the oral environment. This is because the bacterial activity of denture surfaces can be affected by the pH, the presence of certain germs, and saliva.[5,10]

The use of 3D printing technology allows for the creation of customized dentures with tailored antimicrobial properties. Particularly, this could be beneficial for patients with specific oral health needs or those who are more susceptible to denture-related infections.[13] In addition, the use of NPs as antimicrobial agents can help mitigate the development of resistance to conventional antifungal drugs, which has become a growing concern. NPs are effective against a wide range of microorganisms, even at low concentrations.[35]

Limitations of our study were its *in vitro* study nature and small sample size so further studies in clinical settings and with larger sample sizes are needed to validate our findings.

Conclusions

Within the constraints of this investigation, the inclusion of ZrO_2NPs, AgNPs, and TiO_2NPs significantly reduced bacterial count and increased NPs and antibacterial activity. It is suggested that the antimicrobial properties of 3D-printed resin composites are significantly influenced by the kind and concentration of antibacterial agents.

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Conflicts of interest

There are no conflicts of interest.

Author contributions

IBAEAS, EBAE-S, AFE, AAE, and MA: material preparation, data collection, and analysis. IBAEAS:

written the first draft of the manuscript. The manuscript has been read and approved by all the authors, the requirements for authorship as stated earlier in this document have been met, and each author believes that the manuscript represents honest work if that information is not provided in another form. All authors contributed to the study's conception and design. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical policy and Institutional Review Board statement

Not applicable.

Patient declaration of consent

Not applicable.

Data availability statement

Data are available on reasonable requests from the corresponding author.

Abbreviations

TiO_2NP: Titanium dioxide nanoparticles
CAM: Computer-aided manufacturing
CAD: Computer-aided design
ZrO_2NPs: Zirconium dioxide nanoparticles
AgNPs: Silver nanoparticles
UV: Ultraviolet

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