

Antimicrobial Activity of 3D Printed Denture Resin Modified with Three Different Nanoparticles with Various Concentrations (In Vitro Study)

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Received October 24, 2024; Revised November 26, 2024; Accepted December 03, 2024

Abstract Objectives: This study aimed to determine the antibacterial effectiveness of adding zirconia (ZrO₂NPs), silver (AgNPs), and titanium dioxide (TiO₂NP) nanoparticles in various concentrations to 3D-printed denture resin against *C. Albicans*, *S. pyogenes*, and *S. aureus*. **Methods:** Power analysis was used to count the in vitro samples. Using World Health Organization calculations, a research power of 80%, a significance level of 5%, and an error margin of 5% were determined. The antimicrobial efficacy of 150 disk-shaped specimens with a diameter of 15 X 2 mm of unmodified (n= 15) and modified (n= 135) 3D-printed denture resin specimens after the addition of silanated ZrO₂NPs, AgNPs, or TiO₂NPs (n= 45) in varying concentrations (n= 15) of 0.5%, 1%, and 1.5% were compared using three oral bacteria (*Streptococci pyogenes*, *Staphylococcus Aureus*, and *Candida Albicans*) as test subjects. Colony forming units (CFUs) were counted as part of the antimicrobial test. The CFUs count was statistically analyzed using the one-way ANOVA test. Patients subjected to antimicrobial test. **Results:** When the three tested nanoparticles (ZrO₂NPs, AgNPs, and TiO₂NPs) were added, the bacterial count significantly decreased as compared to the unmodified 3D-printed resin, according to the results. Additionally, the findings showed that as the concentration of the studied nanoparticles 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 nanoparticles. **Conclusions:** The antibacterial activity of the resin used in 3D printing was significantly affected by the inclusion of ZrO₂ NPs, AgNPs, and TiO₂NPs.

Keywords: Antibacterial activity, Denture Resin, 3D Printed, Nanoparticles

Cite This Article: Fahad K Alwthinani, "Antimicrobial Activity of 3D Printed Denture Resin Modified with Three Different Nanoparticles with Various Concentrations (In Vitro Study)." *International Journal of Dental Sciences and Research*, vol. 12, no. 4 (2024): 55-60. doi: 10.12691/ijdsr-12-4-2.

1. 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- Comparing computer-aided manufacturing (CAD-CAM), which comprises subtractive methods and additive methods, popularly known as 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 a 3D printing [4,5,6]. The capacity of 3D-printing technologies to immediately receive Computer-aided design (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 pre-existing dentures in addition to the shorter time that operations and lab work require [6,7,8,9].

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,

an antibacterial restorative material would be ideal [11]. The introduction of nanoparticles 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].

Nanoparticles 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, silver, and titanium dioxide nanoparticles with different concentrations to 3D-printed acrylic denture base resins against (*Staphylococcus Aureus*, *Streptococci pyogenes*, and *Candida Albicans*).

2. Materials and Methods

Power analysis was used to count the in vitro samples. Using World Health Organization calculations, a research power of 80%, a significance level of 5%, and an error margin of 5% were determined. For 150 specimens in total, ten groups ($n = 15/\text{per group}$) were made, one group of 3D-printed resin that was left unmodified, and nine groups of resin that had been modified ($n = 135$) using silanated zirconia (ZrO_2NPs), silver (AgNPs), or titanium dioxide (TiO_2NP) (Sigma-Aldrich, USA) with an average granular size of 40 nanometers 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%.

Specimens' Preparation

To improve the bonding between the individual nanoparticles and the resin matrix the various nanoparticles were added after the silane coupling agent had been dissolved in acetone, and the mixture was agitated for 60 minutes, and the acetone was then removed using a rotary evaporator, and the silanized nanoparticles were obtained after cooling. The silanized nanoparticles were introduced to the 3D-printing resin after being weighed on an electronic scale and the fluid resins containing silanized nanoparticles were fully mixed and stirred for 30 minutes using a magnetic stirrer [5]. A disc shape specimen with a diameter of (15 X 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, North Carolina, USA) utilizing digital light printing (DLP). The photopolymerized liquid MMA 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, USA) in an ultrasonic cleaner (Fisherbrand™; ThermoFisher Scientific, 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, 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 minutes [5,15]. (Figure 1)



Figure 1. A Photograph Showing 3d Printed Specimens

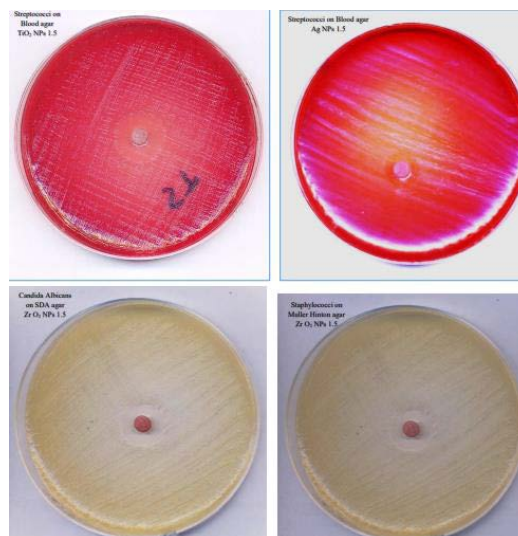


Figure 2. A photograph of agar media with 3D printed specimens

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 milliliters (mL) of brain heart infusion broth culture (BHI) for 24 hours 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 in a 48-well plate with 500 mL of each media and 50 mL of inoculated bacterial suspension for 24 hours [5,11]. The bacterial solution was adjusted to 1.0 X10⁶ colony-forming units (CFUs) /mL for standardization of the bacterial count [11]. Pipette-harvested bacteria from biofilms on discs were diluted to a concentration of 106 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 hours at 37°C [5]. Three times the trials were run with the same outcomes in 107 duplicate. (Figure 2)

Statistical analysis

We assessed the data's normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. A one-way ANOVA test was used to compare the shear and flexural bond stresses. A statistical cutoff point of $P < 0.05$ was employed.

3. 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 ZrO₂NPs, AgNPs, and TiO₂NP 3D-printed resin groups had a significantly higher antimicrobial activity when compared to the unmodified 3D-printed resin (control group). However, for intergroup comparison, the Tukey HSD test results revealed that there was no statistically significant difference between the 0.5% modified ZrO₂NPs, AgNPs, and TiO₂NP 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 ZrO₂NPs and AgNPs 3D-printed resin groups regarding *S. Aureus*, *S. pyogenes*, and *C. Albicans* microorganisms. However, there was a statistically significant difference between the antimicrobial activity between the 1.5% modified ZrO₂NPs, AgNPs, and TiO₂NP 3D-printed resin groups. (Table 1)

The ANOVA results also revealed that the antimicrobial activity significantly increased with the increase of ZrO₂NPs, AgNPs, and TiO₂NP, 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 ZrO₂NPs and TiO₂NPs 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)

Table 1. Comparison of the antibacterial effect at various concentrations

0.5% concentration					
	Control (CFU)	ZrO ₂ NPs (CFU)	AgNPs (CFU)	TiO ₂ NP (CFU)	P-value
<i>S. Aureus</i>	1.0±0.00 ×10 ⁶ Aa	734±65.8 ×10 ³ Ba	740±66.9 ×10 ³ Ba	721± 69.7 ×10 ³ Ba	0.002*
<i>S. pyogenes</i>	1.0±0.00 ×10 ⁶ Aa	726±65.5 ×10 ³ Bb	731±66.8 ×10 ³ Bb	720± 69.9 ×10 ³ Bb	0.0015*
<i>C. Albicans</i>	1.0±0.00 ×10 ⁶ Aa	763±63.9 ×10 ³ Bc	744±49.9 ×10 ³ Bb	756± 82.5 ×10 ³ Bc	0.008*
P-value	1 ns	<0.001*	<0.001*	<0.001*	
1% concentration					
	Control (CFU)	ZrO ₂ NPs (CFU)	AgNPs (CFU)	TiO ₂ NP (CFU)	P-value
<i>S. Aureus</i>	1.0±0.00 ×10 ⁶ Aa	394±47.7×10 ² Ba	396±36.4×10 ² Ba	322± 48.7 ×10 ² Ca	<0.001*
<i>S. pyogenes</i>	1.0±0.00 ×10 ⁶ Aa	383±50.1×10 ² Bb	390±38.1×10 ² Bb	310± 47.0 ×10 ² Cb	<0.001*
<i>C. Albicans</i>	1.0±0.00 ×10 ⁶ Aa	400±41.8×10 ² Bc	418±43.1×10 ² Bb	324± 47.2 ×10 ² Cc	<0.001*
P-value	1 ns	<0.001*	<0.001*	<0.001*	
1.5% concentration					
	Control (CFU)	ZrO ₂ NPs (CFU)	AgNPs (CFU)	TiO ₂ NP (CFU)	P-value
<i>S. Aureus</i>	1.0±0.00 ×10 ⁶ Aa	268±34.7 ×10 ² Ba	351±17.8 ×10 ² Ca	229± 6.5 ×10 ² Da	<0.001*
<i>S. pyogenes</i>	1.0±0.00 ×10 ⁶ Aa	263±37.8 ×10 ² Bb	345±16.9 ×10 ² Cb	223± 5.7 ×10 ² Db	<0.001*
<i>C. Albicans</i>	1.0±0.00 ×10 ⁶ Aa	279±38.9 ×10 ² Bc	370±18.0×10 ² Ca	227± 17.5 ×10 ² Dc	0*
P-value	1 ns	<0.001*	<0.001*	<0.001*	

*: Significant ($p < 0.05$). ns: non-significant. Different uppercase letters mean significant in the same row. Different lowercase letters mean significant in the same column.

4. 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, 3 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]. It was therefore 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 nanoparticles into dentistry was done to improve the material qualities [5].

For material used in 3D printing, nanoparticles might make an appropriate reinforcing approach [4]. To create antibacterial composites, three inorganic antibacterial nanoparticles (ZrO₂NPs, AgNPs, and TiO₂NPs) 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 [11,19,20,21,22,23].

In this current investigation nanoparticles concentrations of 0.5%, 1%, and 1.5% were selected because nanoparticles 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 nanoparticles 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. These results agreed with the results of the previous studies by Chen et al. [11] and Khattar et al. [5] stated that the PMMA resin or photo polymerizes 3D-printed resin had no antimicrobial effect against different tested bacteria.

According to the results of this study, adding ZrO₂ NPs, AgNPs, and TiO₂NPs to the resin used in 3D printing caused a decrease in the number of tested bacterial microorganisms. This might be a result of nanoparticles' antimicrobial action, which has been documented in multiple investigations [5,16,24,25]. The decrease in the number of bacteria could be related to the presence of nanoparticles 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, nanoparticles 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 nanoparticles 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 [16,26]. Moreover, reactive oxygen species that may be produced by nanoparticles and cell membranes interact to enhance cell permeability, which leads to the release of intracellular contents and eventually to cell death [5,16,24,25].

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 nanoparticle concentrations from 0.5% to 1.5%. This may be due to the increased density of ZrO₂ NPs, AgNPs, and TiO₂NPs 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 [5,25,27].

However, the results of this study showed that there was an insignificant increase in the antimicrobial activity of different Ag-NPs when concentration increased from 1.0% to 1.5%. This could be related to the agglomeration of these nanoparticles 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 to low concentration (0.5% and 1.0%) [5,25]. On the other hand, in line with prior work [5,26], the CFU results revealed a negligible reduction in the bacterial count with the addition of nanoparticles in higher concentrations.

The results of this present study revealed that the Ag NPs had a significantly lower antimicrobial effect when compared with the ZrO₂NP and TiO₂NP at 1.5% concentration. These results could be attributed to the higher tendency of Ag NPs to cluster as it was found that Ag NPs 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 nanoparticles, clustered nanoparticles 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 [5,28]. Moreover, the results of this study revealed that the ZrO₂NPs and AgNPs had a lower antimicrobial effect than TiO₂NPs 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 TiO₂NPs, their antibacterial capabilities can be further increased by UV illumination.

To achieve more efficacy with less concentration, 3D printing was preferred [29]. Moreover, this may be a result of TiO₂'s inherent photocatalytic properties to UV irradiation that is used to polymerize the 3D-printed resin. Another restriction on the use of TiO₂ is the quick recombination of photogenerated electron-hole pairs [11]. Since it was identified that UV in 3D printing activates TiO₂'s crystalline form and produces electrons, ROS, superoxide, and OH•, it may be to blame for this effect of the TiO₂ [24].

The results of this present study revealed that there was no statistically significant difference in the antimicrobial activity of each of the ZrO₂NPs, AgNPs, or TiO₂NPs

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) DNA and blocking DNA replication; interacting with the peptidoglycan cell wall and membrane and causing cell lysis [30]. 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. This could be particularly beneficial for patients with specific oral health needs or those who are more susceptible to denture-related infections [13]. Also, the use of nanoparticles as antimicrobial agents can help mitigate the development of resistance to conventional antifungal drugs, which has become a growing concern. Nanoparticles have been found to be effective against a wide range of microorganisms, even at low concentrations [31].

Conclusions

Within the constraints of this investigation, it is suggested that the antimicrobial properties of 3-D printed resin composites are significantly influenced by the kind and concentration of antibacterial agents.

List of Abbreviations

TiO₂NP: Titanium dioxide, **CAM:** Comparing computer-aided manufacturing, **CAD:** Computer-aided design, **ZrO₂NPs:** zirconia, **AgNPs:** silver, **UV:** ultraviolet, **CFUs:** Colony-forming units, **DLP:** digital light printing.

ACKNOWLEDGEMENT

Nill

Financial Disclosure

Not applicable.

Conflict of Interest

The authors have no financial or proprietary interests in any material discussed in this article.

Patient Declaration of Consent

Not applicable

Data Availability

Data is available upon reasonable request from corresponding author.

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