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Research Article

Fabrication of acrylic nanocomposites containing different percentages of TiO2 nanoparticles and their antimicrobial activity compared to other mineral nanoparticles

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ABSTRACT

Considering the novelty of nanotechnology and its applications in dentistry, and the existing conflicts and scarcity of studies regarding the antimicrobial activity of TiO_2, ZnO, and SiO_2 nanoparticles, this study aimed to fabricate acrylic nanocomposites containing different percentages of mineral nanoparticles and assess their antimicrobial activity. In this in vitro, experimental study, standard-strain *Streptococcus mutans* (*S. mutans*) (ATCC35668) and *Candida albicans* (*C. albicans*) (ATCC10231) were cultured The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of zinc oxide (ZnO), titanium dioxide (TiO_2), silver (Ag), and silicon dioxide (SiO_2) nanoparticles were determined by the broth macrodilution and agar well diffusion method. Remarkably, the results revealed that, TiO_2, SiO_2, and ZnO in 1, 0.5, 0.25, 0.125, and 0.062wt% concentrations had no inhibitory effect against *S. mutans* oand *C. albicans*. While, silver nanoparticles (AgNPs) showed in vitro activity against *S. mutans* (MIC=MBC 62.5 µg/ml) and *C. albicans* (MIC 125 µg/ml). However, nanocomposites containing 0.125, 0.25, 0.5, 1, and 2wt% TiO_2 had no in vitro activity against *S. mutans* and *C. albicans*. In the present study, only AgNPs showed antimicrobial activity against *S. mutans* and *C. albicans* in 1, 0.5, 0.25, 0.125, and 0.062wt% concentrations. However, further studies are warranted to correlate these findings with clinical outcomes.

1. Introduction

Auto-polymerizing acrylic resins are mainly composed of polymethyl methacrylate, and are extensively used for the fabrication of removable orthodontic appliances such as expanders, retainers, and functional appliances (Borzabadi-Farahani et al.,2014). Orthodontic appliances interfere with the efficient cleaning of the teeth and oral mucosa by the action of saliva (Hibino et al.,2009). Also, an irregular acrylic resin surface creates a suitable environment for microbial plaque accumulation and changes the oral microbial flora to a cariogenic flora, mainly composed of *Streptococcus mutans* (*S. mutans*) (Türköz et al., 2012).

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On the other hand, Candida stomatitis is a type of oral mucosal inflammation characterized by erythema of the palatal mucosa that more commonly affects the oral mucosa under dentures, removable orthodontic appliances, and retainers (Monteiro et al., 2012; Spampinato et al., 2013). Toothbrushing alone cannot eliminate the microorganisms, and the application of antimicrobial agents is also recommended to remove bacterial biofilm (Jahn., 2010). Nonetheless, regular and efficient brushing of the teeth and orthodontic appliances, and disinfection with antimicrobial agents completely depend on the knowledge, cooperation, and compliance of patients, and are therefore unreliable (Lessa et al.,2007). Thus, researchers have been in search of alternative biocompatible methods independent of patient cooperation to overcome this problem. Accordingly, the addition of antimicrobial nanoparticles to acrylic resins was recently suggested to achieve this goal (Borzabadi-Farahani et al., 2014; Carrouel et al., 2020; Yin et al., 2020). (Sodagar et al., 2016) added titanium dioxide (TiO_2) and silicon dioxide (SiO_2) to acrylic resin and reported a reduction in *S. mutans* and *Lactobacillus acidophilus* counts under in vitro conditions. (Giti et al.,2021) evaluated the antimicrobial activity of TiO_2 and reported that 2.5wt% and 7.5wt% TiO_2 had no significant in vitro activity against *S. mutans*, and only its 7.5wt% concentration had an inhibitory effect on *Candida albicans* (*C. albicans*). (Ghahremanloo et al., 2016) used silver (Ag) nanoparticles (AgNPs) incorporated in acrylic resin and showed that by an increase in its concentration, the in vitro antibacterial effects against *S. mutans* and *C. albicans* increased. (Ghorbanzadeh et al., 2015) in their clinical trial indicated the potent activities of the AgNPs added to the acrylic resin of removable orthodontic appliances on cariogenic microorganisms. (Farhadian et al., 2016) in their clinical trial demonstrated that the deposition of AgNPs on the acrylic surface of orthodontic retainers decreased the count of *S. mutans*. (Yin et al., 2020) also confirmed the antimicrobial activity of AgNPs. Nonetheless, the biocompatibility of AgNPs has not been well confirmed, and some concerns exist regarding the possible risks of the environmental release of AgNPs. (Sadeghi 2014) used Nasturtium Officinale(NO) as a very good eco-friendly and nontoxic source for the synthesis of Ag-NPs. Kachoei et al., 2016 showed the antibacterial activity of zinc oxide (ZnO) nano-coating on nickel-titanium orthodontic wires. (Cierech et al., 2016; Cierech et al., 2016) added ZnO nanoparticles to acrylic resin for the fabrication of a new nanocomposite and demonstrated its optimal antifungal activity against *C. albicans.* They suggested it as an alternative treatment for Candida stomatitis. (Selvarajan et al., 2020) reported the antimicrobial activity of silica nanoparticles and their optimal biocompatibility.

Considering the novelty of nanotechnology and its applications in dentistry, and the existing conflicts and scarcity of studies regarding the antimicrobial activity of TiO_2, ZnO, and SiO_2 nanoparticles, this study aimed to fabricate acrylic nanocomposites containing TiO_2 nanoparticles, and assess their antimicrobial activity compared to SiO_2, Ag, and ZnO nanoparticles.

2. Materials and Methods

This in vitro, experimental study was conducted on acrylic resin blocks containing different concentrations of nanoparticles.

2.1. Fabrication of acrylic blocks:

TiO_2 nanoparticles in 2, 1, 0.5, 0.25, and 0.125wt% concentrations were mixed with acrylic monomer (methyl methacrylate) in a stirrer and sonicated for 30 minutes. After homogenization, the liquid was mixed with polymethyl methacrylate powder (auto-polymerizing acrylic resin; Ivoclar Vivadent, Schann, Lichtenstein) containing glycol methacrylate cross-linker. The mixture was poured into additional silicon (Ormaduplo, Dentari S,p.A) molds to fabricate acrylic discs with 0, 0.125, 0.25, 0.5, 1, and 2wt% concentrations of TiO_2 nanoparticles. The same was performed for other nanoparticles. (Figure 1)

2.2. Microbial tests:

Standard-strain of *S. mutans* (ATCC35668) and *C. albicans* (ATCC10231) were obtained from the Iranian Research Organization for Science and Technology, and were biochemically confirmed. Suspensions were

prepared from TiO_2, SiO_2, ZnO, and AgO nanoparticles in 1, 0.5, 0.25, 0.125, 0.0625, and 0.0312wt% concentrations.

2.3. Determination of minimum inhibitory concentration (MIC) of nanoparticles by the broth macrodilution method:

A total of 2 mL of fresh culture medium (Mitis Salivarius broth for *S. mutans*, and Sabouraud dextrose broth for *C. albicans*) was added to each test tube. Suspensions of TiO_2, SiO_2, AgO, and ZnO nanoparticles in 1, 0.5, 0.25, 0.125, 0.0625, and 0.0312wt% concentrations were then added to the test tubes. In addition, 100 µL of bacterial suspension containing 1.5 x 10^6 colony-forming units (CFUs)/mL was added to each test tube, and the tubes were incubated for 18-24 hours. The positive control test tube contained bacterial suspension without nanoparticles while the negative control contained nanoparticles without bacterial suspension. After incubation, the test tube containing the lowest concentration of nanoparticles with no turbidity (indicating bacterial growth inhibition) was recorded as the MIC.

2.4. Determination of minimum bactericidal concentration (MBC):

To determine the MBC, 50 µL was collected from the contents of the MIC test tube and its previous tube, and cultured on solid medium. After 24 hours of incubation, the respective concentration was recorded as the MBC given that it inhibited the growth and proliferation of 99.9% of the bacteria (Totu et al., 2017).

2.5. Assessment of the antimicrobial activity of nanoparticles by the agar well diffusion method:

After culture of the bacterial suspension of *S. mutans* and *C. albicans* with 0.5 McFarland standard concentration on Mitis Salivarius agar and Sabouraud dextrose agar, some wells were created in the culture plates, and 100 µL of different concentrations of nanoparticles was added to each well. The diameter of the growth inhibition zones was measured after incubation for 18-24 hours(Farhadian et al.,2016).

2.6. Statistical analysis:

Data were analyzed by SPSS version 24.

3. Results

3.1. Inhibitory effects of TiO_2 and AgNPs by the agar diffusion method:

TiO2 did not cause any growth inhibition zone in *S. mutans* and *C. albicans* cultures. The diameter of the growth inhibition zone caused by AgNPs was 18 mm in the *S. mutans* culture and 19 mm in the *C. albicans* culture (Figure 2).

3.2. MIC and MBC:

TiO2 did not cause any growth inhibition of *S. mutans* or *C. albicans*. AgNPs, however, showed a MIC=MBC of 62.5 µg/ml against *S. mutans* (Figures 3 and 4) and 125 µg/ml for *C. albicans* (Figure 5).

3.3. Optical density (OD) results:

The results of the assessment of OD for *S. mutans* and *C. albicans* at 620 nm wavelength are presented in Table 1. The OD was 0.00 for the negative control group and 1.326 for the positive control group for *S. mutans*. The OD was 0.00 for the negative control group and 1.144 for the positive control group for *C. albicans*.

3.4. SiO_2 nanoparticles:

SiO_2 nanoparticles caused no growth inhibition of *S. mutans* or *C. albicans* in the assessment of MIC and MBC.(Figure 6)

3.5. ZnO nanoparticles:

ZnO caused no growth inhibition of *S. mutans* or *C. albicans*.(Figure 7)

3.6. Antimicrobial activity of acrylic resin containing TiO_2 nanoparticles:

None of the tested concentrations showed any activity against *S. mutans* and *C. albi*cans in the MIC test. (Figure 8)

Table 1. OD for *S. mutans* and *C. albicans* at 620 nm wavelength

Test tube number	*S. mutans*		*C. albicans*	
	AgNPs	TiO_2	AgNPs	TiO_2
1	1.226	2.723	1.390	2.984
2	0.926	2.408	0.486	2.515
3	0.352	2.078	0.365	2.201
4	0.91	1.815	0.452	1.954
5	1.170	1.700	1.036	1.933
6	1.520	1.509	1.451	1.864

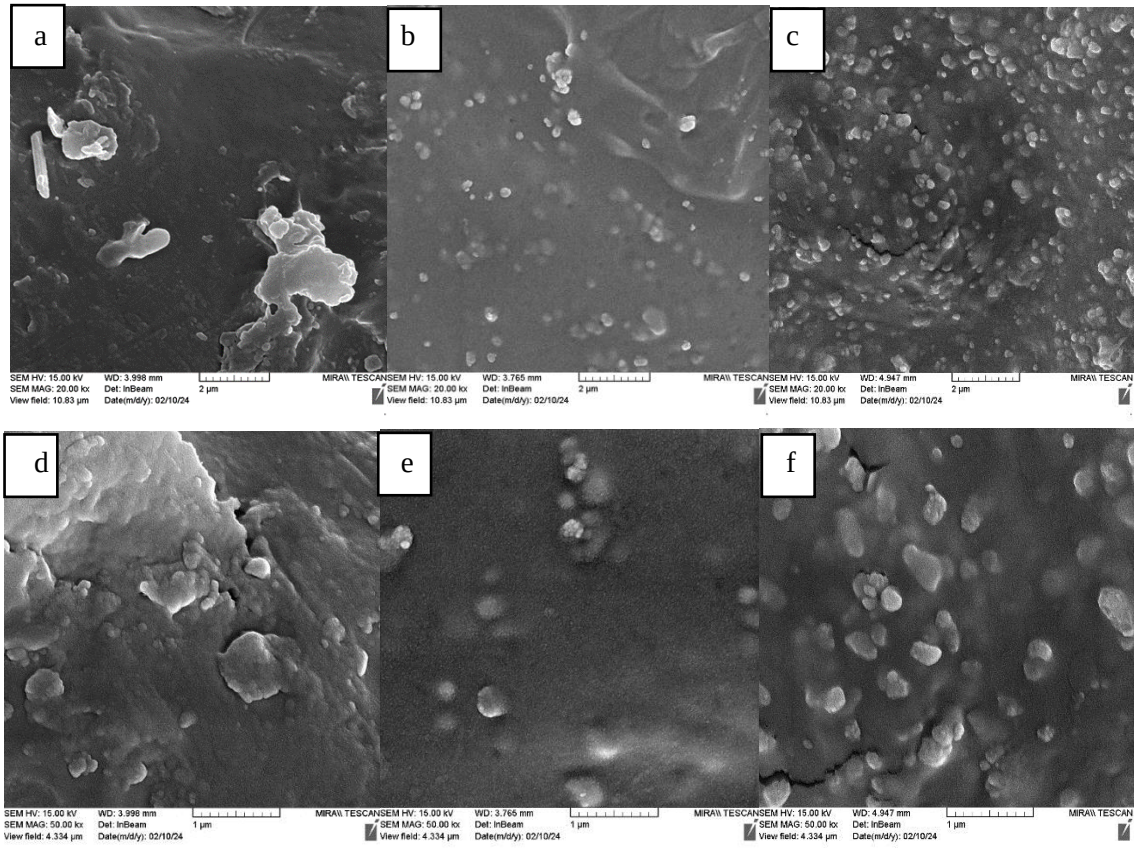

Figure 1: Field emission scanning electron microscopy(FE-SEM) images of a,b,c acrylic resin with 0, 0.5% and 2wt% nanoparticles can be seen in 1µm magnification, respectively, and d,e,f acrylic resin containing 0, 0.5% and 2% TiO2 nanoparticles are shown in 2µm magnification, respectively.

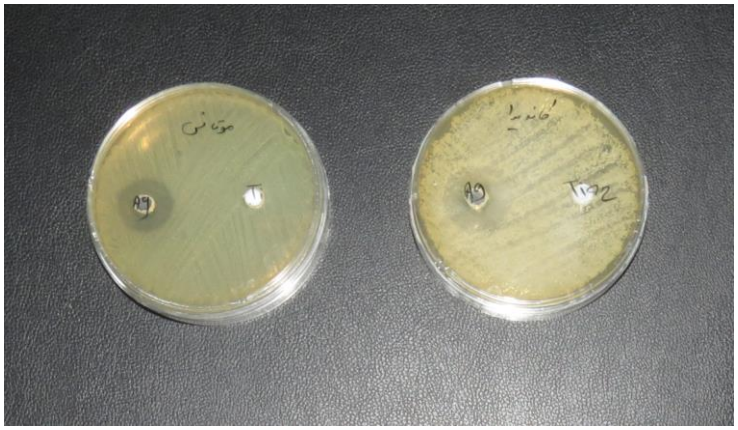

Figure 2. Growth inhibition zones caused by TiO_2 and AgNPs in *S. mutans* (left) and *C. albicans* (right) cultures

Figure 3. MIC of AgNPs for *S. mutans*. The concentration decreases from left to right, and turbidity was eliminated after the third test tube.

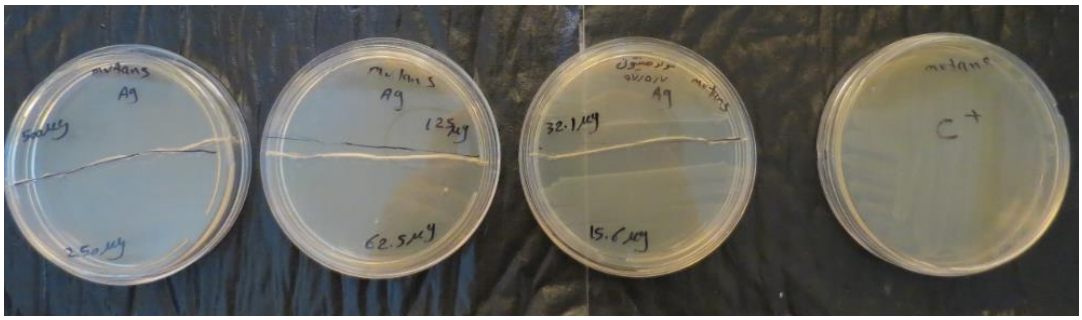

Figure 4. MIC and MBC of AgNPs for *S. mutans* in Mueller Hinton agar

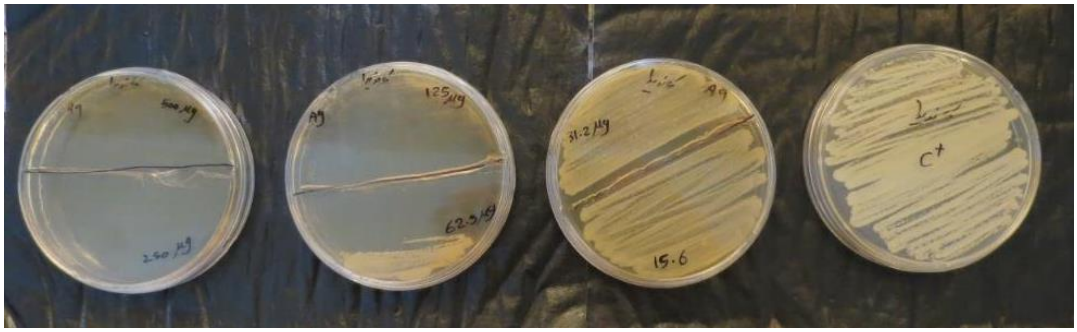

Figure 5. MIC and MBC of AgNPs for *C. albicans* in Mueller Hinton agar

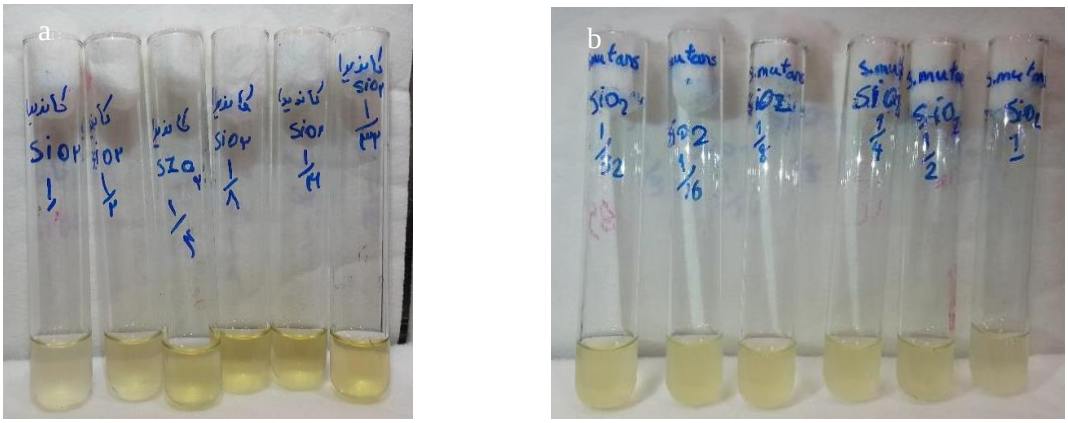

Figure 6. MIC of SiO_2 for *C. albicans* (a), and *S. mutans* (b)

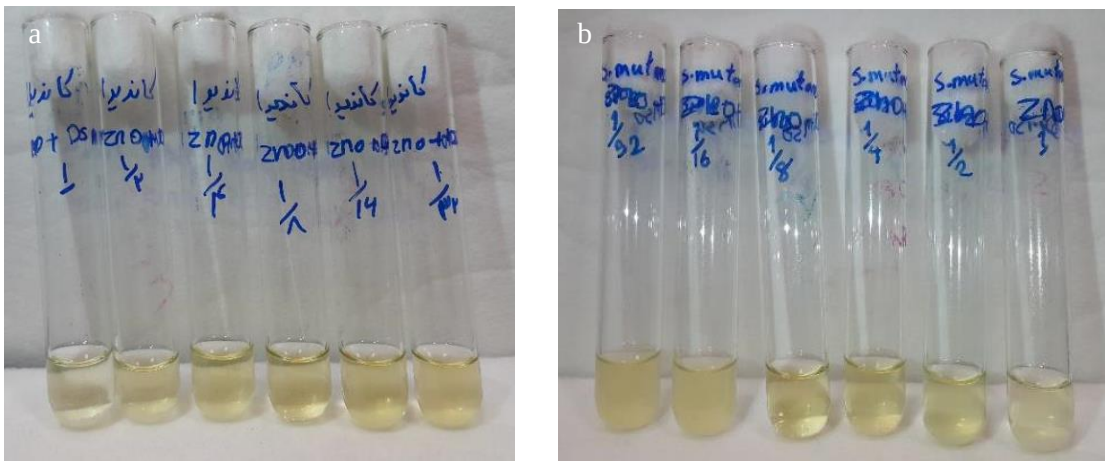

Figure 7. MIC of ZnO for *C. albicans* (a), and *S. mutans* (b)

Figure 8. MIC of TiO_2 nanocomposites for *S. mutans* (a), and *C. albicans*(b)

4. Discussion

In the present study, acrylic nanocomposites containing TiO_2, SiO_2, Ag, and ZnO nanoparticles were fabricated and their antimicrobial activity was assessed. TiO_2, SiO_2, and ZnO nanoparticles in 1, 0.5, 0.25, 0.125, and 0.062wt% concentrations had no inhibitory effect against *S. mutans* and *C. albicans*, and turbidity was seen in all concentrations. AgNPs showed MIC=MBC of 62.5 µg for S. mutans and 125 µg for *C. albicans*. TiO_2 nanocomposites in different concentrations had no inhibitory effect on *S. mutans* or *C. albicans*. (Giti et al., 2021) showed no significant effect of 2.5 and 7.5wt% TiO_2 on *S. mutans*; however, 7.5wt% TiO_2 significantly inhibited *C. albicans*, compared with the control group. Thus, it appears that low concentrations of TiO_2 nanoparticles that have no significant effect on the physical properties of acrylic resin have no inhibitory effect on S. mutans and C. albicans; a higher concentration of 7.5wt% only had an inhibitory effect on C. albicans. (Giti et al., 2021) also demonstrated significant antimicrobial effects of both concentrations of TiO_2 on Streptococcus sanguinis, Streptococcus salivarius, and Candida dubliniensis, which were not investigated in the present study. (Totu et al., 2017) indicated that acrylic resin containing 0.4, 1, and 2.5% TiO_2 nanoparticles had inhibitory effects on Candida *scotti* using the DHA toxicity control method. They showed that by an increase in the concentration of TiO_2 nanoparticles, the mechanical properties of acrylic resin decreased. Thus, the concentration of nanoparticles can only be increased to some extent. Their results could not be compared with the present findings since their methodology and type of microorganism were different from those of the present study. Unlike (Totu et al., 2017; Alrahlah et al., 2018) demonstrated an improvement in nano-mechanical properties of acrylic resin such as hardness and modulus following an increase in the concentration of TiO_2 nanoparticles from 1% to 3wt%. Unlike the present study, (Alrahlah et al., 2018) counted the Enterococcus faecalis and Pseudomonas aeruginosa colonies and found that by an increase in the concentration of TiO_2 nanoparticles from 1wt% to 3wt%, the colony count significantly decreased. (Sodagar et al., 2016) suggested UV radiation improve the antimicrobial properties of polymethyl methacrylate containing TiO_2 and SiO_2 nanoparticles. They showed that UV irradiation maximized the antimicrobial efficacy of polymethyl methacrylate containing TiO_2 and SiO_2 nanoparticles.

As mentioned earlier, the present study revealed that AgNPs had a MIC=MBC of 62.5 µg for S. mutans, and 125 µg for C. albicans.

Consistent with the present results, Takamiya et al., 2021; Yin et al., 2020; Suganya et al., 2014; Li et al., 2016; Ahmad et al., 2020 showed the antifungal properties of AgNPs added to acrylic resin against *C. albicans*.

In the present study, different concentrations of SiO_2 had no inhibitory effect on S. mutans or C. albicans. Sodagar et al., 2016 suggested UV radiation improve the antimicrobial properties of polymethyl methacrylate containing SiO_2. Another study introduced AgNPs incorporated in spherical mesoporous SiO_2 nanoparticles as a potent antifungal agent against C. albicans and showed their concentration-dependent antifungal activity. They used a combination of AgNPs and SiO_2 nanoparticles (Qasim et al., 2015). In line with the present results, they demonstrated that AgNPs had inhibitory effects on C. albicans but SiO_2 had no antifungal effect in tested concentrations.

Future studies are required on the antimicrobial effects of higher concentrations of TiO_2, SiO_2, ZnO, AgNPs, and also other nanoparticles. Implication of novel and nontoxic techniques for synthesis of NPs (Sadeghi et al., 2014; Khodadad et al., 2021) is suggested. Moreover, the mechanical properties of nano-composites containing nanoparticles should be investigated.

Conclusion

In the present study, only AgNPs showed antimicrobial activity against S. mutans and *C. albicans* in 1, 0.5, 0.25, 0.125, and 0.062wt% concentrations. TiO_2, SiO_2, and ZnO nanoparticles in 1, 0.5, 0.25, 0.125, and 0.062wt% concentrations had no inhibitory effect against *S. mutans* and *C. albicans*, and turbidity was seen in all concentrations. AgNPs showed MIC=MBC of 62.5 µg for S. mutans and 125 µg for *C. albicans*. TiO_2 nanocomposites in different concentrations had no inhibitory effect on *S. mutans* or *C. albicans*. However, further studies are warranted to correlate these findings with clinical outcomes.

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Refereces

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