ORIGINAL RESEARCH

Effect of Adding Silver Nanoparticles on the Flexural Strength of Feldspathic Porcelain

Farnaz Firouz[1], Fatemeh Amiri[2], Sara Khazaei[3], Fariborz Vafaee[4], Abbas Farmany[5], Maryam Farhadian[6]

Abstract

Aim: This study aimed to evaluate the impact of silver nanoparticles (AgNPs) on the flexural strength of feldspathic porcelain.

Materials and methods: Eighty bar-shaped ceramic specimens were prepared in five groups, including a control group and four case groups containing 5, 10, 15, and 20% w/w of AgNPs. Each group consisted of 16 specimens. Silver Nanoparticles were synthesized by a simple deposition method. Three-point bending test was used in the universal testing machine (UTM) machine to evaluate the flexural strength of the specimens. The fractured surface of the ceramic samples was analyzed under scanning electron microscopy (SEM). In order to analyze the data obtained, one-way analysis of variance (ANOVA) and Tukey tests were used ($p < 0.05$).

Results: The results implied that the average flexural strength of the samples in the control group was 90.97 MPa and for the experimental groups reinforced with 5, 10, 15, and 20% w/w of AgNPs were 89, 81, 76, and 74 MPa, respectively.

Conclusion: The addition of AgNPs with a certain amount (up to a concentration of 15% w/w) without reducing the flexural strength improves the antimicrobial properties of the materials used and ultimately improves its quality for dental applications.

Clinical significance: The addition of AgNPs can improve the antimicrobial properties and suitability of the materials.

Keywords: Feldspathic porcelain, Flexural strength, Nanoparticles.

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Introduction

Nanotechnology is defined as the design and fabrication of materials at the atomic and molecular levels. The National Nanotechnology Initiative's strictest concept of nanotechnology refers to structures with at least a single dimension in the 1–100 nm size range.[1]

The introduction of nanotechnology to the dental sciences has caused an obvious improvement in this field such as caries prevention, and improvement of physical and antibacterial properties.[2,3] Nanomaterials have a high ratio of surface to volume, so they provide a higher surface for reaction compared to other materials. For example, these materials react with the membrane of the bacteria and generate their antimicrobial effect in a large surface.[4] It has been reported that nanoparticles provide a higher level of physical, chemical, and mechanical properties compared to other microparticles; therefore, they can be used for the synthesis of dental materials with high mechanical properties and better antimicrobial effects.[5]

AgNPs have recently received a lot of attention because of their superior physical, chemical, and biological properties. Their superiority stems primarily from their size, shape, composition, crystallinity, and structure once compared to their bulk forms. There have been initiatives to investigate their alluring qualities and make use of them in useful applications, including antibacterial and anticancer therapeutics, water disinfection, diagnostics and optoelectronics, and other clinical/pharmaceutical ones. Silver is an inexpensive and widespread natural resource with intriguing material properties.[6]

The antibacterial properties of AgNPs have been studied more than other nanoparticles. The application of silver compounds in restorative materials, endodontic cements, dental implants, and caries prevention solutions.[4,5] In the study of Thangavelu et al., it has been reported that AgNPs, due to their unique antimicrobial and antiviral properties, can be used in various fields of dentistry, such as endo, perio, prosthetic, restorative, etc.[7]

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Dental ceramics are widely used due to their desirable beauty, durability, and excellent biocompatibility. However, several drawbacks such as reduced plastic deformation, brittleness, low fracture resistance, and reduced impact toughness have limited their applications, especially in posterior teeth.[8,9] Porcelain's physical properties are altered when nanoparticles are added, and the materials' mechanical properties are also impacted such as strength, elasticity, and density as well as improve the performance quality of the materials.[9] Karthikeyan et al. showed that the addition of AgNPs to feldspathic porcelain increases the toughness of these materials.[10]

The results of Sasikala and Chander study showed that the addition of zirconia nanoparticles to feldspathic porcelain increases their flexural strength.[9] So far, a few studies have been conducted on the impact of AgNPs on the porcelain's flexural strength. In this study, AgNPs are included primarily for their antimicrobial function. It is anticipated to have an impact on the investigated porcelain's flexural strength. For this reason, a simple deposition method was used to synthesize AgNPs from pomegranate peel extract. Moreover, the antibacterial activity and cytotoxicity of the prepared materials were investigated. The effects of adding different amounts of AgNPs on the flexural strength of feldspathic porcelain were also investigated.

MATERIALS AND METHODS

Study Design

Eighty bar-shaped ceramic specimens were divided to five groups; each group consisted of 16 specimens (Fig. 1) as:

1. Group A (control group): Noritake feldspathic porcelain without AgNPs
2. Group B: Noritake feldspathic porcelain with 5% w/w of AgNPs
3. Group C: Noritake feldspathic porcelain with 10% w/w of AgNPs
4. Group D: Noritake feldspathic porcelain with 15% w/w of AgNPs
5. Group E: Noritake feldspathic porcelain with 20% w/w of AgNPs

Sample Preparation

In order to prepare the ceramic samples approximately 4 mm in width, 1.2 mm thickness, 25 mm in length, a resin pattern with the mentioned dimensions was prepared, and then for each group, 16 ceramic bars were prepared using a polyvinyl siloxane mold ($n = 16$).

Silver Nanoparticles synthesized were added to pure distilled water using carboxymethyl cellulose for uniform particle distribution. The porcelain paste was applied to a mold with a wet brush after the powder and molding liquid were combined. To get rid of extra water, the samples were carefully dried in an oven. After drying for 5 minutes, they were placed in a furnace (VITA VACUMAT 6000 M, Zahnfabrick, Bad Säckingen, Germany) with an increase in temperature of 55˚C/minute, vacuum rate of 730 mmHg, the initial cooking temperature of 500˚C for 30 minutes, and finally cooled. Eventually, the ceramic samples were polished with 320, 600, 1200, and 2400 particle size sandpaper, respectively, and the thickness of the samples was checked using a digital caliper. The resulting ceramic samples were purified in distilled water with ultrasonic (EUROSONIC 4D-made in Italy). A UTM was used to measure the flexural strength. Before each measurement of the sample strength, the device was first calibrated according to the factory instructions.

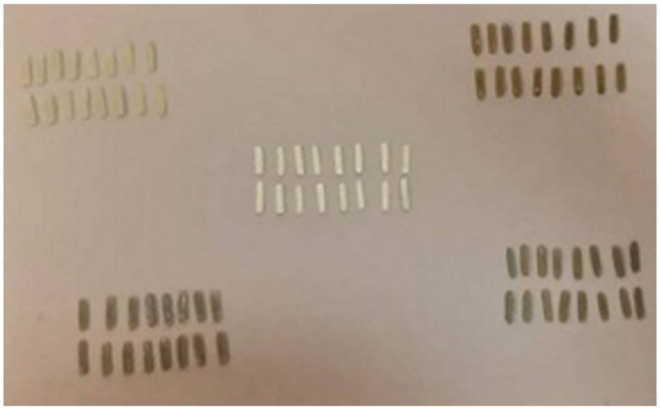


Fig. 1: Samples (80 bar-shaped ceramic specimens)

Three-point bending test was used in the UTM machine with a speed of 1 mm/minute to evaluate the flexural strength of the samples. The samples were placed between two hardened steel supports, and a determined force was applied to the center of the samples. The force required to break each sample was measured in Newtons and converted to flexural strength in MPa in accordance with the following equation:

$3WL/2BD^2$
W: The force required to break the sample
L: Distance between two supports
B: Sample width
D: Sample thickness

The mean flexural strength for each group was derived. The fractured surface of the ceramic samples was analyzed under SEM and under specific magnification to identify the dispersion of particles and voids. Figure 1 show groups containing 5, 10, 15, and 20% w/w of AgNPs, respectively.

Synthesis of Silver Nanoparticles

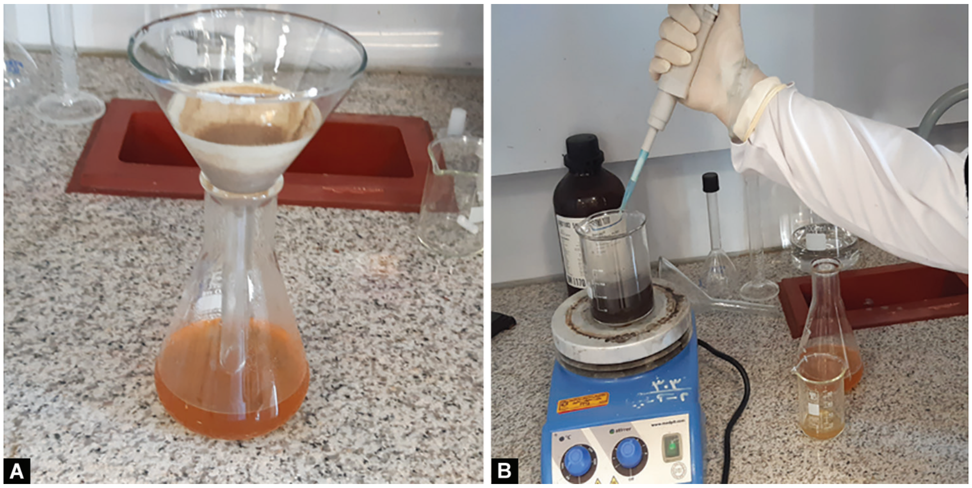
Silver Nanoparticles were synthesized using Propolis. Propolis is a natural bee product with flavonoid components and phenolic content. Commercially available propolis powder was purchased from Bazar Hamadan (Iran). In order to prepare an ethanolic extract of propolis, 50 gm of the powder was placed in 500 mL of 70% v/v ethyl alcohol at room temperature for 72 hours. Then, the extract was separated by filtration with Whatman Grade 40 ashless filter paper (Fig. 2A). In order to completely separate the suspended fine particles of powder from the prepared extract, a centrifuge was used. In order to prepare AgNPs, a solution of silver nitrate (10 µg/mL) was prepared. After adjusting its pH to 9.5, the prepared propolis extract (0.1 mg/mL) was slowly added to the silver nitrate solution and placed at 70°C, and stirred for 2 hours (Fig. 2B). The change in color of the solution from light yellow to brownish yellow indicated the synthesis of AgNPs. The precipitate obtained in this step was washed with double-distilled water and centrifuged (4000 RPM-Behdad-Iran).[11]

The following methods were used to characterize the nanoparticles:

- X-ray diffraction (XRD): In this method, a Panalytical Xpert PRO X-ray Diffractometer (Panalytical, Netherlands, Xpert Pro MPD model) with a wavelength of 1.5405 Å and a power of 40 KV/30 mA was used to investigate the crystal structure of the nanoparticles.
- Transmission electron microscopy (TEM): An electron microscope was applied to evaluate the surface morphology and size distribution of the nanoparticles.

Cell Viability Assay

Fibroblastic cells (L929 cell line was purchased from Cell bank of Pasteur Institute of Iran) with a density of ~1×10^4 cells were cultured in a 96-well plate and exposed to different samples with different coating of 5, 10, 15, and 20% w/w of AgNPs (sintered porcelain loads with AgNPs) under the following conditions: RPMI medium with 10% fetal bovine serum (FBS), 50 mL/IU penicillin and 50 µg/mL streptomycin, and incubated at 37°C and 5% CO_2 in the atmosphere. A group without treating was nominated as control group. All experiments were performed triplicates. The MTT



Figs 2A and B: Synthesis of AgNPs

Table 1: Mean and standard deviation of the flexural strength in the studied groups

Group	*Number*	*Mean*	*SD*	*Min.*	*Max.*	*p-value*
Flexural strength						
Control A	16	90.9752	21.29924	67.68	121.76	0.016
B 5% w/w of AgNPs	16	89.6418	19.62982	51.61	123.24	
C 10% w/w AgNPs	16	81.3887	13.89516	62.21	101.96	
D 15% w/w AgNPs	16	76.0906	15.54253	45.49	102.84	
E 20 % w/w AgNPs	16	74.0617	14.70931	48.22	94.94	

assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. In this experiment, the treated cells and also the control group after 48 hours were incubated with MTT solution for 3 hours. Briefly, the cell media were removed and cells were washed three times with phosphate buffered saline (PBS) is a balanced salt solution used for a variety of cell culture applications, such as washing cells before dissociation, transporting cells or tissue, diluting cells for counting, and preparing reagents. Then, MTT solution (50 µL) was added, and after 3 hours of incubation, subsequently, dimethyl sulfoxide (DMSO) is an organosulfur compound. It is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water was added to each well to dissolve, and then after 10 minutes of shaking, an enzyme-linked immunosorbent assay (ELISA) is a commonly used analytical biochemistry assay reader (Lab system Multiscan) was used to read at 580 nm. All experiments were performed in triplicates.

Antibacterial Activity Assay

The standard bacteria of *Streptococcus mutans* (PTCC 1683) strain was obtained from the Iranian research organization for science and technology. In order to evaluate the antibacterial activity of the AgNP-modified porcelain, after exposure of the bacteria (*S. mutans*) to the AgNPs-modified porcelain, 200 µL of each suspension treated with a micropipette was spread on a nutrient agar plate overnight and then incubated at 37°C. After 24 hours, the colonies of the control and exposed plates were counted by light microscopy. Without treating nominated on a group (as the control group), all experiments were performed in triplicates.

Statistical Analysis

Data were analyzed using SPSS (version 20.0). In order to analyze the data, the Kolmogorov–Smirnov test was used to assess normality. Due to the normal distribution of variables in different groups, one-way ANOVA was used for analysis. In order to compare the two groups, the Tukey *post hoc* test was used.

RESULTS

The results of mean values and standard deviation of the flexural strength in different groups are shown in Table 1.

The result of the one-way analysis of the variance test was significant for the flexural strength variable (p-value = 0.016). Therefore, in order to compare the two groups, the Tukey *post hoc* test was used (Table 2).

The results of the Tukey's test showed that the difference between the control and E group (porcelain with 20% w/w of AgNPs) was statistically significant. The box diagram for comparing the flexural strength among groups is shown in Figure 3.

Characterization of AgNPs

The XRD pattern of the synthesized AgNPs is shown in Figure 4. The major peaks observed at 2θ of 32.34, 38.14, 43.26, 66.25, and 73.85 correspond to the (111), (200), (220), and (311) Miller indexes, respectively.

Figure 5 represents the TEM image of the used AgNPs. TEM analyses indicated that colloidal AgNPs with a diameter between 20 and 50 nm were obtained during the synthesis process.

Table 2: Comparison of groups in terms of the flexural strength variable

Variable	*Group*	*Group (J)*	*SE (I-J)*	*SD*	*p-value*
Flexural strength	Control (A)	B	1.08339	5.95927	1.000
		C	9.33645	5.95927	0.523
		D	14.63458	5.95927	0.112
		E	16.66345	5.95927	0.049*
	B = 5% w/w AgNPs	C	8.25306	5.95927	0.639
		D	13.55119	5.95927	0.165
		E	15.58006	5.95927	0.078
	C = 10% w/w AgNPs	D	5.29813	5.95927	0.900
		E	7.32700	5.95927	0.734
	D = 15% w/w AgNPs	E	2.02887	5.95927	0.997

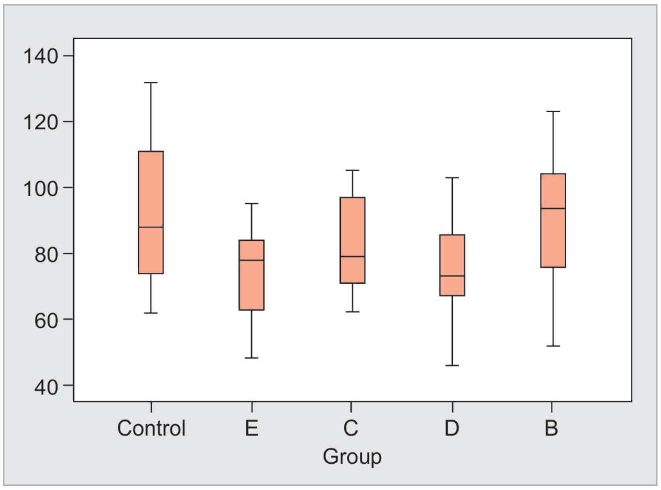


Fig. 3: Box diagram for comparing flexural strength between groups

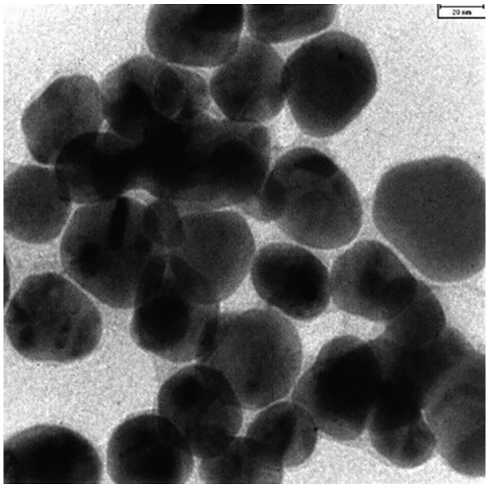


Fig. 5: TEM image of AgNPs (scale bar: 20 nm)

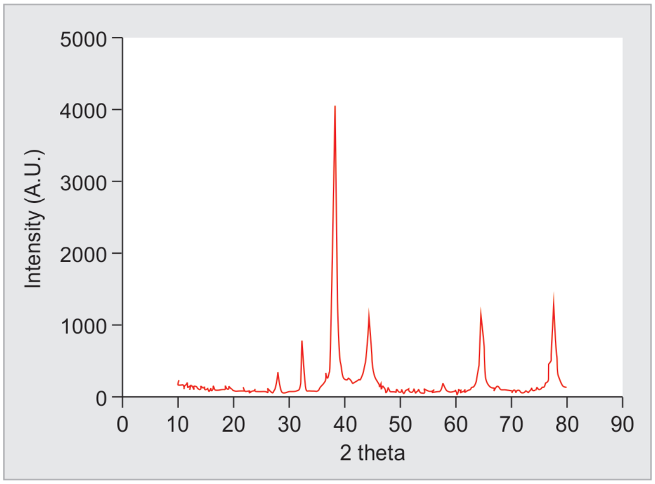


Fig. 4: XRD pattern of AgNPs

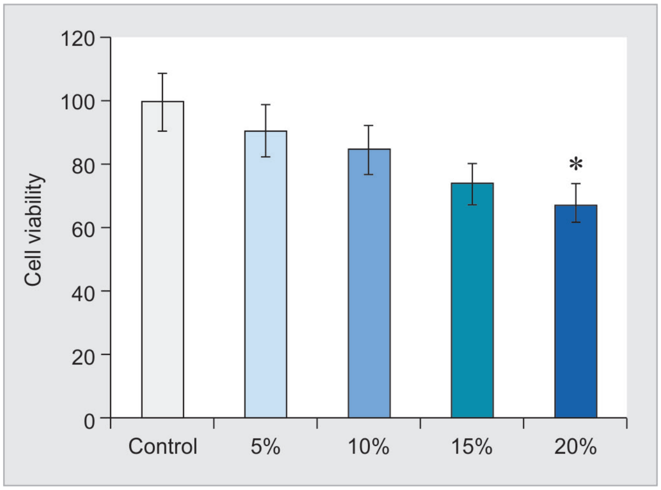


Fig. 6: Results of cell viability test. *p-value <0.05

Biocompatibility Assay

The biocompatibility results of this study showed that the viability of L929 cells decrease by nanoparticles addition. The cell survival rates of 100%, 91 ± 10%, 85 ± 9%, 74 ± 9%, and 68 ± 8% were obtained for the control, 5, 10, 15, and 20% w/w of AgNPs, respectively. Although the cell viability in the samples treated with AgNPs decreased compared to the control group, this decrease was statistically significant only in the 20% w/w group ($p \leq 0.05$, Fig. 6). The results of this study showed that the samples are compatible.

Antibacterial Activity Assay

As demonstrated in Figure 7, there was a decrease in the number of colonies formed in nanoparticle-modified porcelain samples. The number of colonies per group studied (control, 5, 10, 15, and 20% w/w of AgNPs) was 101, 84, 68, 49, and 27, respectively. The decrease in the number of colonies was significant in all groups compared to the control group ($p \leq 0.05$).

DISCUSSION

This study was performed to investigate the impact of adding AgNPs on the flexural strength of feldspathic porcelain. The samples were made according to the ISO 6872 standard and evaluated using three-point bending test by a UTM device.

Since 1980, ceramics have been widely used, including inlays, onlays, veneers, and fixed prostheses with short lengths. With the development of new materials with remarkable mechanical properties, ceramic implants, abutments, and posts were synthesized. Over the past 30 years, the increasing demand of patients for restorations with natural and beautiful appearance has led to the development of all-ceramic materials that their mechanical properties have been significantly improved.[12]

Silver Nanoparticles

Metal nanoparticles with diameters fewer than 100 nm have had a significant effect on a wide range of biomedical applications, including diagnostic and therapeutic devices. AgNPs were widely used in a variety of medical applications, including imaging probes, antimicrobial agents, coatings for medical devices, drug and gene carriers, and optoelectronics. Recent research has centered on quick, green, and environment-friendly development techniques for the synthesis of AgNPs as cost-effective synthesis methods.[13–17]

Several studies have been conducted on the positive effects of adding AgNPs to porcelain, and it has been shown that, in addition to antimicrobial properties, many physical and mechanical properties such as toughness and fracture resistance have been improved. For this reason, we used AgNPs in our study.

Antimicrobial Effect and Biocompatibility of Silver Nanoparticles

It appears that bacteria are far less likely to acquire resistance against metal nanoparticles than other conventional and narrow-spectrum antibiotics. This is thought to occur because metals may act on a broad range of microbial targets, and many mutations should occur for the microorganisms to resist their antimicrobial activity.[18]

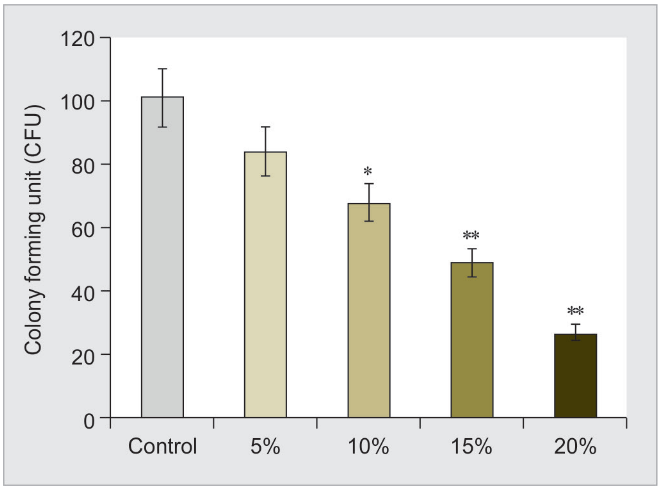


Fig. 7: Number of colonies formed in the studied groups. *p-value <0.05, **p-value <0.001

Leakage margin of veneers means loss of adhesion and flooding of the surface between the tooth and restoration, which results in tooth decay, pulp sensitivity, necrosis, periodontal disease, cosmetic problems, and endangering the lifetime of the restoration. Therefore, its length needs to be improved dental restoration lifetime that is combined with biocompatible agents to combat microbial degradation and recurrent caries while maintaining occlusal load tolerance. The antimicrobial effects of silver ions have always been well known since ancient times. These ions have high antibacterial effects against 16 species of bacteria. The antimicrobial effect of AgNPs is associated with the formation of free radicals and subsequent destruction of cell membranes. The antiviral and antimicrobial effects of these particles have been proven in various studies. According to Hashem et al., adding AgNPs and silver hydroxyapatite to porcelain was a very effective technique in preventing the formation of bacterial colonization.[19] The antibacterial activity test conducted in the current study revealed that all added concentrations of AgNPs to feldspathic porcelain significantly increased the antibacterial activity of the obtained porcelain. As shown in Figure 7, the number of colonies decreased after the addition of AgNPs, and the largest decrease was related to the group treated with 20% w/w of AgNPs.

The solution color in this investigation changed from light yellow to brownish-yellow as a result of the synthesis of AgNPs using PPE that is consistent with recent relevant findings.[20,21] The recorded 2θ XRD peak of the synthesized AgNPs can be seen in Figure 4. The resulting XRD pattern demonstrated the formation of cubic AgNPs crystals. TEM of biosynthesized nanoparticles presented their clear morphology with a spherical form from 30 to 50 nm (Fig. 5).

The results of biocompatibility and antimicrobial effect of AgNPs modified porcelain in the present research are along with the findings of Kim et al. In their study, it was found that the addition of AgNPs to porcelain mixture *in vitro* increased osteoblast cell activity and led to a significant antibacterial activity against *S. mutans* bacteria (especially porcelain containing 30% w/w of AgNPs).[22]

Effect of AgNPs on Properties of Dental Ceramic

Due to the antimicrobial properties of AgNPs, various studies have studied their effect on other properties of ceramics. Karthikeyan et al. found that the samples reinforced with titanium and AgNPs showed significant increases in fracture toughness, and the blending of superior concentrations of both titanium and AgNPs decreased fracture resistance.[10] Uno et al. indicated that the addition of AgNPs significantly increased the fracture toughness and Vickers hardness of the Noritake Super porcelain.[23]

Ferreira et al. showed that the addition of b-$AgVO_3$ nanoparticles in all added concentrations showed antibacterial effects but no effect on Vickers microhardness, and the 10% by mass group (c group) had higher roughness than the other groups.[24]

The addition of AgNPs increases the fracture toughness of ceramics. Given that silver has a higher thermal expansion coefficient than the glass matrix, this increase in fracture toughness might be caused by this. The compressive stress in the tangential direction prevents cracks from expanding toward the silver particles, leading to crack deflection. On the contrary, the addition of silver hydroxyapatite nanoparticles decreased the fracture strength values. This decrease could be due to interference in the porcelain firing process because of particle aggregation. However, the addition of both of these nanoparticles has a negative effect on the color of the tooth ceramic.[25] Similar results have been reported by other researchers in which the addition of hydroxyapatite in a higher percentage reduces the strength of dental porcelain.[26]

Fujieda et al. studied dental porcelain with AgNPs for its effects on static fatigue. This parameter increased with increasing concentration of AgNPs that might be effective in inhibiting the fatigue fractures of dental porcelains in the oral cavity.[27] The mechanical qualities of porcelain were improved by the inclusion of silver and platinum nanoparticles, including Young's modulus and the fracture toughness. The presence of these nanoparticles drastically influences the porcelain's color.[28]

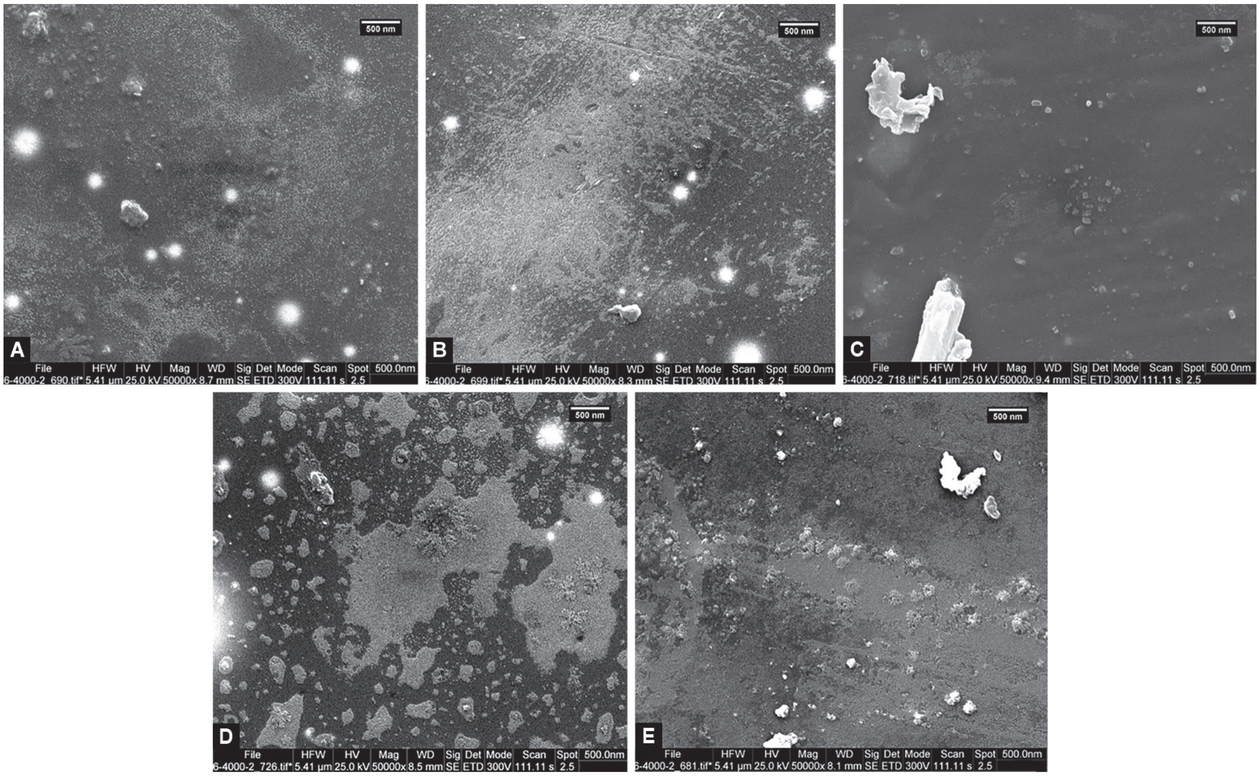
Regarding the effect of AgNPs on porcelain color, Firoz et al. showed that adding AgNPs to VitaVM9 feldspathic porcelain up to 500 gm/mL did not cause discoloration over the clinically detectable threshold ($\Delta E = 2.69$) or increase ceramic translucency. The results of the biocompatibility test and antibacterial activity of AgNPs modified porcelain were consistent with our results. Moreover, it was found that in the porcelain samples containing biocompatible AgNPs, the antibacterial activity of the samples increased with increasing nanoparticle concentration.[29]

Flexural Strength

Sasikala and Chander showed that the addition of zirconia nanoparticles to feldspathic porcelain increased their flexural strength due to the dispersion strengthening mechanism. Because of the higher surface energy of the nanoparticles, the nanopowder tends to accumulate, and if they were distributed evenly, they would tend to improve density and strength. However, the addition of these nanoparticles to lithium disilicates reduced the flexural strength. The reason for this reduction may be attributed to the nanoparticles that did not fuse into the glass matrix of the lithium disilicate structure.[9] The result of this study is a little different from our conclusion. In our study, the average flexural strength of the control group was obtained to be 90.97 MPa and for the experimental groups reinforced with 5, 10, 15, and 20% w/w of AgNPs, which was obtained to be 98, 81, 76, and 74 MPa, respectively. The results showed that the flexural strength was significantly different between the four studied groups, and its rate was higher in the control group compared to the other groups. Side-by-side comparison showed that the amount of flexural strength in the 20% w/w group was significantly lower than the control group. Adding 20% w/w of nanoparticles adversely affected the mechanical properties.

Sasikala and Chander showed that the reinforced feldspathic ceramic has an increase in strength with reinforcement. The nanozirconia-integrated feldspathic had improved the strength of ceramic due to the dispersion strengthening mechanism. Then, nanopowder has a strong agglomeration tendency due to its high superficial energy, and they tend to increase the density if they were uniformly distributed.[16] The nanozirconia reinforcement hindered the crack propagation through the material, increased the density of the sintered samples when pressed, and thus improved the strength of reinforced feldspathic samples. It seems that the difference arises from the type of ceramic. They used pressable feldspathic porcelain unlike us.[9]

Tah et al. studied feldspathic porcelain with zirconia-silica nanofibers for its effects on the flexural strength. The flexural strength of samples reinforced with 5 and 7.5% wt zirconia-silica nanofibers was higher than the control group. The zirconia-silica



Figs 8A to E: The SEM of the groups. (A) Control; (B) 5% w/w of AgNPs; (C) 10% w/w of AgNPs; (D) 15% w/w of AgNPs; (E) 20% w/w of AgNPs nanofibers reinforced feldspathic ceramics have the property of bridging the crack area, which further prevents crack propagations. So, it could be said that the mechanism of this increase, and a little difference from our results, was due to the shape of the nanoparticles.[30]

SEM

The results of SEM analysis showed that the glass matrix of the feldspathic porcelain structure had not been fused with the nanopowders, despite the fact that they functioned as a weak site with growing porosity and decreased the structure's strength (Fig. 8). The utilized nanoparticle cannot be absorbed and cannot function as a nucleating agent, and hence the presence of nanoparticles in the porcelain matrix has inhibited the crystallization of porcelain crystals.

The SEM of the control group showed a uniform glass matrix (Fig. 8A). In the group, the broken surface of the samples had irregular areas between the large smooth areas of the glass matrix (Fig. 8E). These irregularities were attributed to the presence of heterogeneous clusters scattered among the glass matrix, and their morphology was dendritic.

The studied groups showed clusters of silver fine particles scattered among the areas of the glass matrix. There were many dense masses on the fractured surface of the glass matrix, which revealed that nanoparticle penetration had changed over time in the crack's plane of propagation and caused the production of cracking steps. The experimental groups showed an increase in the quantity of surface porosity and a heterogeneous matrix. The nanopowders may act as a weak site by increasing porosity and decreasing the strength of the generated ceramics.

The nanopowders have a high tendency to agglomerate due to their high surface energy, which led to agglomeration, bubble formation, nonuniform stress transfer, and reduced toughness.[12]

There are several ways to prevent agglomeration, such as layer-by-layer technique, spark plasma sintering method, and dynamic method of incorporating silver, which can be used to increase the material's properties. It has been reported that plasma spark sintering and nanoparticle composition dynamic methods can be effectively used to improve the properties of materials.[9]

This study was limited to just one brand of porcelain, and it should be noted that *in vitro* conditions are dramatically different from *in vivo* conditions.

CONCLUSION

The results of this study showed that adding AgNPs to feldspathic porcelain up to a concentration of 15% w/w does not reduce flexural strength. The antibacterial activity test findings revealed that the addition of AgNPs increased the feldspathic porcelain's antibacterial properties. Moreover, the cell biocompatibility test showed that the porcelain samples containing AgNPs are biocompatible. Therefore, it can be concluded that the addition of AgNPs with a certain amount (up to a concentration of 15% w/w) without reducing the flexural strength improves the antimicrobial properties of the materials used and, as a result, their suitability for dental applications.

It is suggested to evaluate the probable effects of aging on mechanical properties and optical behavior of porcelain containing nanoparticles in the future studies. In addition, the clinical significance of the potential toxicity of AgNPs remains unknown; thus, further studies are essential because clinical evidences are still limited.

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