Antimicrobial properties of tissue conditioner containing silver doped bioactive glass nanoparticles: *in vitro* study

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Abstract

The objective of this work is to prepare and verify the antimicrobial properties of tissue conditioner containing silver doped bioactive glass nanoparticles (Ag-BG NPs) *in vitro* against three oral pathogens. A novel bioactive glass (BG) nanoparticles (NPs) with different Ag weight percentages of 0.6, 1.2, 3 and 4 wt% was prepared via a modified sol-gel method. Characterization of Ag-BG NPs was carried out using transmission electron microscopy (TEM), Fourier transformer infrared spectrophotometer (FTIR). Antimicrobial activities of the NPs were estimated. Also, Ag⁺ ions release was measured using an inductively coupled plasma optical emission spectroscopy (ICP/OES). The 4% Ag-BG NPs was incorporated into the tissue conditioner (TC) for the preparation of the composite specimens (TC/Ag BG 4%). The dispersion of the NPs was evaluated using SEM/EDX. Ag⁺ ion release and antimicrobial activities were evaluated. Finally, cell cytotoxicity was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on normal human fibroblast (BJ1). Data analysis was performed using one-way ANOVA, and Tukey’s post hoc test. The Ag-BG NPs (4%) exhibited the highest statistical significant mean inhibition value against selected pathogens. A novel Ag-BG NPs with antimicrobial properties with gradual release of Ag⁺ were developed. However, the antimicrobial activity was lost when it was incorporated into the tissue conditioner.

Keywords: silver doped bioactive glass nanoparticles, tissue conditioner, antimicrobial properties, cell cytotoxicity

Classification numbers: 2.04, 4.02, 5.08
1. Introduction

The use of soft liners is an indispensable aid in the treatment of patient wearing removable prostheses. Being resilient, soft liners compensate the reduced thickness of oral mucosal and equilibrate the masticatory stresses; thus, minimizing the incidence of pressure points, traumatic ulcers and pain during mastication [1, 2]. Furthermore, their application is recommended in cases requiring maxillofacial prosthesis; As they alleviate patient’s pain and discomfort without causing trauma to the soft tissues which is sensitive and easily irritated [3].

Two types of soft liners are available: permanent liners and temporary liners which are known as tissue conditioner. Currently, most of the available permanent liners made of silicon and methyl/ethyl methacrylate cross-linked amorphous polymers [4]. On the other hand, tissue conditioners consist of un-crosslinked amorphous polymers. They are supplied in the form of powder and liquid. The main constituent of the powder is poly (ethyl methacrylate) or a related copolymer. The liquid is a mixture of a plasticizer as butyl phthalyl, butyl glycolate, dibutyl phthalate, dibutyl sebacate, and ethyl alcohol. Other material based on poly (butyl methacrylate) or a related copolymer does not contain ethyl alcohol in the liquids [5].

One of the major problems associated with the use of soft liners is inevitable biofilm formation and subsequent microbial colonization [6]. Microorganisms as Candida albicans (C. albicans), Streptococcus mutans (S. mutans) and Staphylococcus aureus (S. aureus) have been isolated from patients wearing removal prostheses [7]. Such pathogens are capable of initiating infectious diseases as denture stomatitis, pharyngeal and respiratory infections particularly in elderly and medically compromised individuals [8–11]. Although, different mechanical and chemical cleansing methods are available, none of them were efficient to prevent biofilm formation or to eradicate microorganisms from lining materials. Besides, these methods can cause substantial damage to liners affecting their durability [12]. Therefore, the maintenance of integrity of soft liners and the prevention of the microbial accumulation are of great importance, the search for liner with antimicrobial characteristics is an important demand for the maintenance of removable prostheses [13].

Silver is well-known for its antimicrobial characteristic against a broad spectrum of bacteria and fungi [14]. With the development of nanomaterials, silver nanoparticles (AgNPs) demonstrated potent antimicrobial characteristics for treating infectious diseases including drug-resistant ones [15, 16]. One of the critical aspects of silver NPs in biomedical practice is its precipitation that could result in toxic effects. Therefore, the need for silver carrier as glass nanoparticles is desirable prerequisite. Silver doped glasses (Ag-BG) are reported to be very effective bactericidal agents compared to those containing silver in other oxidation states (±3) [17, 18]. The present study aimed to investigate the biocidal activity of tissue conditioner containing silver doped bioactive glass nanoparticles against three pathogens: Candida albicans, Streptococcus mutans and Staphylococcus aureus.

2. Materials and methods

2.1. Preparation and characterization of Ag-doped bioactive glass nanoparticles

Novel Ag-doped bioactive glass nanoparticles (Ag-BG NPs) with four weight percentages of Ag ions were prepared via a modified sol-gel method [19]. Silver oxide (Ag2O) was added to glass composition at the expense of the calcium oxide (CaO) as expressed by the general formula: 80SiO2 - (20 - x)CaO - xAg2O, all in wt% and x = 0.6, 1.2, 3 and 4%. The prepared glass compositions were represented in table 1. The particle size was studied using transmission electron microscopy (TEM, JEM-HR-2100, Japan). The infrared spectra were carried out using a Fourier transformer infrared (FTIR) spectrophotometer (model FT/IR-6100 type A, USA). Nitrogen adsorption-desorption isotherms were determined by means of a high-speed gas sorption analyzer (NOVA 2000 series, Chromatic, UK). The Brunauer–Emmet–Teller (BET) method was employed to determine the specific surface areas. In addition, pores volume, average pore diameters and pore-size distribution were also estimated.

2.2. Determination of antimicrobial activities of Ag-BG NPs

The antimicrobial characteristics of Ag-BG NPs were assessed using well diffusion method.

2.2.1. Microorganisms. The microorganisms tested were: C. albicans (ATCC10231), S. aureus (ATCC6538) and S. mutans. The latter were isolated and identified by the Microbiology Lab, Department of Microbiology and Dairy Science, National Research Centre, where S. mutans was obtained by swabbing all surfaces of the teeth of high caries index patient (National Research Centre ethical committee approval registration no: 13-028), using sterile cotton tipped swab (Q-tips, Dermacea, Sherwood Medical, St Louis, USA). The swab was placed in a 5 ml sterile container containing 2 ml of phosphate-buffered saline (PBS, pH 7.4). Then swab in PBS was placed in a vortex (Thermolyne Maxi Mix II, Iowa, and USA) for five minutes to dislodge bacteria followed by Serial dilutions to form dilution at 10−1, 10−2 and 10−3 using an automatic micropipette. The selective media mitis salivarius-bacitracin agar (MSB, Fluka and catalogue No. 01337, Sigma-Aldrich, USA) was used to isolate and grow S. mutans.

Each of S. aureus and S. mutans were grown in test tubes containing 10 ml of treptone soya broth (TSB, Difco, Detroit, MI USA). Then, they were incubated at 37°C for 24h to grow. Each culture was diluted into fresh media until a concentration of 1 × 106 CFUml−1 was reached and used as a working microbial solution [20].

For the C. albicans, the fungal strain was kept on sabouraud broth medium (1% peptone, 4% glucose and adjusted to pH 5.8) to grow at temperature range of 35–37°C for 48 h. Then, culture was diluted into a fresh media until a concentration 1 × 106 CFUml−1 was reached and used as a working microbial solution.
2.2.2. Microbiological assay. Aqueous suspensions of the four glass samples (Ag-BG 0.6, Ag-BG 1.2, Ag-BG 3 and Ag-BG 4) were prepared (200 mg/ml) [21]. For S. mutans, S. aureus, and C. albicans, 20 µl of each working microbial solutions was spread evenly over a nutrient agar plate. Then, six wells were cut using sterile 5 mm cork-borer. Four of these wells were filled with 50 µl of the previously prepared Ag-BG suspensions (200 mg/ml in water). The fifth well was filled with 50 µl saline water as a negative control and the sixth one was filled with 50 µl of mycostatin (Nystatin 100 000 units/ml-1 suspension, Glaxo Smith Kline, Giza, Egypt) as a positive control against C. albicans, while 0.2% chlorhexidine digluconate (CHX, Sigma Aldrich, USA) was used as a positive control against S. mutans and S. aureus [22].

Following incubation period specified for tested strains, the plates were examined visually for the inhibition zones. Each assay was repeated three times independently (triplicate). Ag-BG NPs compositions that showed the highest inhibition zone values was selected and used for the preparation of the Ag-BG tissue conditioner composite specimens.

2.3. Ag⁺ ions release from Ag-BG 4 NPs powder

Ag⁺ ions released from Ag-BG 4 sample was evaluated using an inductively coupled plasma optical emission spectroscopy (ICP/OES, Model Agilent 5100VDV, Germany) with a detection limit of 0.01 mg/l-1. 50 mg of Ag-BG 4 NPs was immersed in 100 ml of deionized water in polypropylene tubes at 37°C under agitation for different periods: 2 h, 4 h, 24 h, 48 h, 4 days, 5 d, 6 d, 7 d, 8 d, 9 d and 10 d.

2.4. Preparation of tissue conditioner (TC) composite containing the Ag-BG 4 (TC/Ag BG 4%)

Tissue conditioner (Co soft, GC, USA) was supplied in the form of powder and liquid. For the preparation of the composite discs, different weight ratios (3%, 4%, 6%, 12.5%, 25% and 35%) of Ag-BG 4 NPs were used. Ag-BG 4 NPs particles were added to the monomer liquid in order to reduce the agglomeration of the NPs and to ensure its appropriate dispersion [23]. Disc specimens (20 x 4 mm²) were prepared by adding the powder to liquid (6 gm:5 ml) then manually mixed following the manufacturers’ instruction. Mixture was packed into specifically designated metal molds, sandwiched between two glass slabs and allowed to set. Ag-BG 4 NPs 35% weight ratio was the proper to be added without affecting consistency of the tissue conditioner.

A total of 19 composite disc specimens were prepared. All specimens were sterilized using ultraviolet light to prevent the bacterial contamination [24]. Similarly, six tissue conditioner disc specimens were prepared and used as control. The specimens used for Ag⁺ was kept immersed in deionized water.

The microstructure of the cross-section of composite containing Ag-BG 4 was examined by means of SEM/EDX (SEM Model Quanta FEG 250, Holland) to confirm the presence of the Ag-doped glass nanoparticles within its matrix.

2.5. Evaluation of antimicrobial activity of composite

The antimicrobial activity of the composite and its elutes (leach out) were evaluated against same strains using agar disk diffusion [25] and well diffusion methods respectively. Specimen elutes materials were obtained by keeping the specimens immersed in 100 ml of double deionized water for two days, two weeks and four weeks at 37°C in a shaking incubator (Innova 43, New Brunswick, USA).

Using the agar disk diffusion method, 20 µl of each previously prepared working microbial solution were spread evenly over a nutrient. A 5 mm filter paper discs (Whatman, USA) containing 0.2% chlorhexidine and mycostatin (positive control) were prepared. The filter paper discs and composite specimens were placed onto the agar surface. For elutes, well diffusion method was used. Four wells were cut. Three wells were filled with 50 µl of elutes material and the fourth one was either filled with 50 µl of 0.2% chlorhexidine digluconate or the mycostatin. The agar plates were incubated and cultured following the same procedures as described before for the tested strains. The diameters of inhibition zones formed around specimens, elutes and positive control were measured in mm.

2.6. Determination of Ag⁺ ions release from TC/Bag4 composite

In polypropylene tubes, composites were immersed in 100 ml of deionized water at 37°C under agitation. The released Ag⁺ ions were measured after two days, two weeks and four weeks using an inductively coupled plasma optical emission spectroscopy (ICP/OES, Model Agilent 5100VDV, Germany).

2.7. Cytotoxicity

The potential cytotoxicity effect of TC/BGA4 composite was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay test. The test was conducted on human normal fibroblast cell (BJ1) in Bioassay-Cell Culture Laboratory (National Research Centre, Cairo, Egypt). All test steps were performed in a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA).

2.7.1. Sample preparation for cytotoxic test. Both composite and control specimens (tissue conditioner only) were immersed in 100 ml of Dulbecco’s modified eagle’s medium

Table 1. Different compositions of bioactive glass in weight%.

| Sample  | Bioactive glass compositions (wt%) |
|---------|----------------------------------|
|         | SiO₂ | CaO | Ag₂O |
| Ag-BG 0.6 | 80   | 19.4 | 0.6  |
| Ag-BG 1.2 | 80   | 18.8 | 1.2  |
| Ag-BG 3   | 80   | 17   | 3    |
| Ag-BG 4   | 80   | 16   | 4    |
Figure 1. TEM images of the Ag-doped bioactive glass nanoparticles: (a) Ag-BG 0.6, (b) Ag-BG 1.2, (c) Ag-BG 3, and (d) Ag-BG 4.

Figure 2. FTIR spectra of (a) Ag-BG 0.6, (b) Ag-BG 1.2, (c) Ag-BG 3 and (d) Ag-BG 4.
Ham’s F-12 (DMEM/F12) cell culture medium (1:1 MIX Lonza, Veries, Belgium) for 2 d and 4 weeks. Cytotoxicity of the specimen elutes were assessed, after each immersion period. Specimens were examined in three independent tests.

2.7.2. MTT assay. BJ1 cells were suspended in DMEM/F12, 1% antibiotic-antimycotic mixture (10,000 U ml\(^{-1}\) potassium penicillin, 10,000 µg ml\(^{-1}\) streptomycin sulfate and 25 µg ml\(^{-1}\) amphotericin B) and 1% L-glutamine at 37 °C under 5% CO\(_2\). Cells were batch cultured for 10 d and seeded at concentration of 10 × 10\(^3\) cells/well in a new growth medium into 96-well microtiter plastic plates at 37 °C for 24 h under 5% CO\(_2\) using water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA).

After 24 h, the media was replaced with equal volumes of elute materials from both composite specimens’ and tissue conditioning. For negative control, cells were incubated in DMEM/F12 alone. Whereas, positive control 100 µg ml\(^{-1}\) of a natural agent of known cytotoxic effects (100% lethality) under the same conditions [26, 27] was used.

After 48 h of incubation, the medium was replaced with 40 µl MTT salt (2.5 µg ml\(^{-1}\)) and incubated for further 4 h at 37 °C under 5% CO\(_2\). To stop the reaction and dissolve the formed crystals, 200 µl of the 10% sodium dodecyl sulphate (SDS) in deionized water was added then incubated overnight at 37 °C. Absorbance was measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between specimens and negative control using independent \(t\)-test by SPSS 11 program. The percentage of change in viability was calculated according to the following formula:

\[
\text{Change in viability (\%)} = \left( \frac{\text{Reading of specimens}}{\text{Reading of negative control}} - 1 \right) \times 100.
\]

Figure 3. \(N_2\) adsorption-desorption isotherms measured for all Ag-doped bioactive glass nanoparticles: (a) Ag-BG 0.6, (b) Ag-BG 1.2, (c) Ag-BG 3 and (d) Ag-BG 4.
2.8. Statistical analysis

Data were tabulated as mean (M) and standard deviations (SD). For microbial assays and ion release test statistical analysis were performed using IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) statistics version 20 for windows. One-way analysis of variance (ANOVA) was followed by Tukey’s post hoc test for multiple comparisons and P value was set at P < 0.05. Regarding cytotoxic effects independent t-test was used.
3. Results

3.1. Characterization of Ag-doped bioactive glass nanoparticles (Ag-BG NPs)

3.1.1. Transmission electron microscopy. TEM images of all glass compositions are presented in figure 1. Cluster of glass particle of sizes less than 100 nm was revealed. Hence the nanosized particles were proved to be formed.

3.1.2. Fourier transformer infrared spectrophotometry. The FTIR spectra of the Ag-BG 0.6, Ag-BG 1.2, Ag-BG 3 and Ag-BG 4 are shown in figures 2(a)–(c) and (d), respectively. The bands sited within the range of wavenumber 1000–1200 cm\(^{-1}\) were associated to the Si–O–Si asymmetric stretching vibration, whereas the bands found in the range of 725–800 cm\(^{-1}\) were linked to the Si–O–Si symmetric stretching vibration. Furthermore, the bands detected in the range of (450–480) cm\(^{-1}\) were correlated to the Si–O–Si bending mode [19].

3.1.3. Textural analysis. The textural properties of Ag-BG NPs determined from the nitrogen (N\(_2\)) adsorption and desorption isotherms were demonstrated in figure 3. Corresponding to the IUPAC the glass compositions were identified as type IV, which is descriptive of the mesoporous materials of 2–50 nm [28]. The hysteresis loop of this isotherm revealed different paths for desorption and adsorption branches at relatively high pressure \(P/P_0\).

The pore size distribution curves obtained for all Ag-doped glass nanoparticles (Ag-BG 0.6, Ag-BG 1.2, Ag-BG 3 and Ag-BG 4) by employing the density functional theory (DFT) method were presented in figures 4(a)–(c) and (d) respectively. All samples show narrow and mono-modal pore size distribution in the mesoporous range. The specific surface area (SSA) was calculated using Brunauer–Emmett–Teller (BET) method. The textural characteristics of the Ag-doped glass nanoparticles are listed in table 2.

3.2. Antimicrobial activity

3.2.1. Antimicrobial activity of Ag-BG NPs. The glass nanoparticles samples (Ag-BG 1.2, Ag-BG 3 and Ag-BG 4) showed antimicrobial activity against tested strains (tables 3–5). On other hand, the Ag-BG 0.6 sample could not induce an inhibition zone. The NPs were more efficient against \(S.\) mutans followed by \(C.\) albicans and at last the \(S.\) aureus.

3.2.2. Antimicrobial activity of composite specimens. The Ag-BG NPs 4% specimens did not show any inhibitory effect against the selected strains, while the positive control (0.2% chlorhexidine digluconate) induced an inhibition zone.
The concentration Ag-BG 4 demonstrated the highest mean values against the three test stains compared to other Ag-BG NPs and it was statistically significant ($P < 0.05$).

### 3.3. Characterization of TC/Ag-BG 4 composite

Figure 5 shows the SEM/EDX micrograph of Ag-BG 4 composite cross-section. It demonstrates that the glass NPs were distributed throughout the matrix. EDX analysis revealed silica, calcium and silver peaks which are the main components of the glass NPs.

### 3.4. Silver ions release

The release of the Ag$^+$ from the Ag-BG 4 NPs sample was measured up to the eighth day attaining the maximum amount in the fourth day ($11.5 \text{ mg l}^{-1}$) as shown in figure 6. Afterwards, there was a gradual decrease in the Ag$^+$ concentration with detection limit of (0.01 mg l$^{-1}$).

In contrast, the composite specimens showed gradual Ag ion release till the end of the fourth weeks (figure 7). Statistical analysis revealed that there was no significant difference between two days and two weeks ($P > 0.05$), but there was a significant difference after four weeks ($P < 0.05$).

### 3.5. Cytotoxicity

Tissue conditioner (TC) showed no cytotoxic effect after two days, but there was negligible reduction in cell viability after 4 weeks (2.46%). This difference was statistically significant ($P < 0.05$). On the other hand, the composite (TC/Ag-BG 4) shows slight reduction in cell viability after two days (5.5%) and four weeks (7.96%) and it was statistically insignificant ($P > 0.05$). Comparing the effect of TC and composite TC/Ag-B on the cell viability, there was statistical significant difference after two days ($P < 0.05$) and insignificant difference after four weeks ($P > 0.05$). From this result (table 6) it is apparent that Ag-BG 4 is nontoxic (biocompatible) as their cell viability 92%.

### 4. Discussion

Ag NPs is one of the most extensively investigated nanomaterial for its bactericidal and fungicidal characteristics as reported in several previous and recent studies [29–32].

Several studies reported incorporation of Ag NPs in polymeric dental materials such as denture base materials, soft liners and orthodontic adhesives [33–36]. Although, it adds antimicrobial benefit, the optimal quantity of Ag NPs is crucial, to avoid the cytotoxic risks owing to the precipitation of the Ag NPs [37]. Bioactive glasses nanoparticles containing Ag$^+$ NPs could be a better alternative to Ag NPs alone as Ag$^+$ ions form a fundamental part of the glassy matrix.

In the present study the antimicrobial activity of the prepared Ag-BG NPs powder was tested against three oral pathogens: C. albicans, S. mutans and S. aureus. The formation of inhibition zones confirmed the antimicrobial activity of bioactive glass containing Ag$^+$ and the increased antimicrobial activity was concurrent with the concentration. Such finding denotes the release of Ag$^+$ from the glass particle surface [38], its dissemination into the agar media and its inhibitory effects upon tested pathogens growth. Similar antimicrobial findings exhibited by the glass NPs containing ZnO was reported in the literature [21]. The release of Ag from the bioactive glass of 4% concentration was further confirmed using the ion release test. Although the maximum Ag ions release value was detected in the fourth day, there was a gradual release till the eighth day. Consequently, it was proved that the bioactive glass affords a controlled release of Ag$^+$ ions by time in the presence of moisture up to a certain limit [39].

The Ag-BG 4 was the most effective antimicrobial weight percentage; therefore, it was chosen to be added to the tissue conditioner in order to prepare the composite specimens. Even though, other studies supported the antimicrobial or inhibitory potentials of tissue conditioner or denture base resin containing Ag NPs [33–36, 40]. In the present study no inhibitory or antimicrobial effects were detected in tissue conditioner containing the Ag-BG 4 NPs.

This outcome could be attributed to the fact that Ag$^+$ is bonded inside the polymer matrix thus, limiting its release, accordingly reducing its fungicidal and antibacterial activities [41] despite the fact that the Ag peak was apparent in the

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**Table 6. Cytotoxicity activity test.**

| Time       | Control (tissue conditioner) (%) | Composite specimen TC/BG 4 (%) | $P$-value |
|------------|----------------------------------|-------------------------------|-----------|
| After 2 d  | zero                             | 5.5 ± 3.54                    | AS        |
| After 28 d | 2.467 ± 1.55                     | 7.96 ± 4.572                  | 0.1198*   |

AS: absolute significance.

* Insignificant difference.
elemental analysis (EDX). This is in harmony with Green et al [42] who pointed out that inhibition zone diameter is not related only to the level of antimicrobial efficiency, but similarly to the diffusion of the antimicrobial agent into the media and to the total quantity of agent available to diffuse. This finding is in agreement with a previous study conducted by Wady et al [35] who observed that AgNPs added to denture acrylic resin did not induce antifungal activity and only AgNPs aqueous solution showed antimicrobial activity against tested pathogens. The concentration and surface area of AgNPs incorporated into the polymer [43], and the characteristics of the polymer base may also affect the leaching of Ag [43, 44].

In the current study Ag\(^+\) release test was performed to ascertain the leaching out of Ag\(^+\) from tissue conditioner (TC) composite specimens at different intervals. Ag\(^+\) release was detected till second day (48 h) with a minimal detection limit of 0.01mg/l\(^-1\). Interestingly, after fourth day there was a gradual increase in the amount of Ag\(^+\) release reaching maximum within four weeks with obvious change in the specimens’ color. Several factors may explain these findings: the presence of wet environment as deionized water [45], alteration of Ag ions into metallic Ag, and the release of silver into the surrounding media which is influenced by the dynamic dissolution of the glassy matrix in the liquid media. Consequently, glass may act as a dispenser for Ag\(^+\) providing a precise release of Ag\(^+\) which is very important to maintain the biocidal activity for a long time [39].

Another possible explanation for this remarkable increase of released Ag\(^+\) is that: the initial release of Ag\(^+\) was from the NPs at the surface while more time was required for Ag\(^+\) released from NPs deeply located the polymer matrix to migrate to the surface of the specimen [45]. Besides, water diffusion, allows polar groups present in the polymeric chains to be separated, and the water dipoles are then attached to those of the tissue conditioner polymer. Thus, the polymeric structure is re-assumed with water incorporation. Accordingly, a free volume is created in the polymer, which could permit molecular flexibility, ion and particle movement [44, 45]

5. Conclusions

Within the limitations of this in vitro study the following was concluded:

1. Novel silver doped bioactive glass nanoparticles with different silver concentrations were developed.
2. The three different concentrations of silver doped bioactive glass NPs (Ag-BG 1.2, Ag-BG 3 and Ag-BG 4) showed antimicrobial activity against selected pathogens.
3. Silver doped bioactive glass NPs, provides gradual release of Ag\(^+\) as a matter of time.
4. Tissue conditioner containing silver doped bioactive glass NPs and its elutes does not possess any antimicrobial properties or cytotoxic effects, however, Ag ions release increased gradually with time attaining maximum value after four weeks.

5. Further studies are suggested to reach the optimum percentage of Ag in bioactive glass to formulate (obtain) tissue conditioner with antimicrobial characteristics.

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