1. Introduction

Bioactive glass has been extensively used as a bone filler and bone graft material due to its unique ability to bond with natural bone. The material creates bonds through ion exchange in simulated body fluid (SBF) after a series of chemical reactions. This bond capability gives bioactive glass a wide range of biomedical applications [1]. Many researchers are working to enhance this material bioactivity and to develop many more aspects such as its antibacterial properties also and its cytotoxicity [2–4]. The researchers have focused their attention on bioglass ceramics which are formed through sol-gel synthesis and have SiO$_2$, CaO and P$_2$O$_5$ as major components. In the sol-gel synthesis, precursors are used to make and collect nanoparticles into a gel form at room temperature. The glass obtained through this method after drying and heating of the gel achieves high homogeneity [5]. Various techniques were investigated to enhance the bioactivity of the biomaterials inside the physiological environment by introducing various metal ions into the glass matrix. Researchers have developed silver-doped bioglass enhancing the bioactivity and functionality of various metallic ions such as strontium, boron, copper, silver, magnesium and titanium [6–8]. Among these various metal ions, strontium (Sr) has some particularly interesting properties, such as enhancement of bone stability in the direction of osteogenesis. Strontium is also very useful in the treatment of osteoporosis [9,10]. Incorporation of strontium ion into the glass matrix acts as a stimulator to improve the bone properties [11]. Strontium (Sr) is also known to reduce bone resorption and boost damaged bone recovery processes [12]. Researchers provided large numbers of facts about the mechanism of movement of strontium in bone cells [13]. It was reported that osteoporosis is a primary cause of bone density loss and that the addition of strontium into bioglass facilitates stimulates bone formation and also increases its antioxidant properties [14]. Bioactive glass doped with strontium shows the superior quality and advanced development of bone minerals for Sr-doped glasses compared with Sr-free glasses [15]. Strontium helps to stimulate new bone and tissue formation and reduces bone resorption [16–19]. Strontium is a bone-seeking element and its dose concentration affects bone metabolism. At low dose levels, strontium contributes significantly to new bone formation and biomaterials containing strontium are excellent for bone repair applications [20–22].

In this investigation of bioactive glass compositions containing silver was introduced into the glass matrix because of its antibacterial activity. Antimicrobial agents based on silver content are attracting considerable interest because of the low toxicity of active Ag$^+$ ions to human cells and because silver also have high...
ions have a broad antibacterial spectrum and limited toxicity to the human body [26]. There are however partial studies on strontium containing bioactive glass bone substitutes in which silver ions act as antibacterial agent. The introduction of strontium into the glass matrix also helps to enhance cell proliferation. An in-vitro time-dependent study was carried out on pure glass 46S6 and Sr-doped glass 46S6Sr10, and the results obtained from this study reveal that 0.1 wt% of Sr induces enhancement of cell proliferation by about 14.3% compared to pure glasses [27]. We need to extend the investigation to consider SiO₂-CaO-SrO-Ag₂O in-vitro behavior of nanoscale strontium containing bioglass accompanied by doping with silver as an antibacterial agent. We synthesized for the first time a new bioactive bioglass composition of the system: SiO₂-CaO-SrO-Ag₂O by sol-gel route and studied its structural behavior, bioactivity and antimicrobial properties with appropriate analytical techniques. The main focus of this research was concerned adding strontium along with silver into the glass matrix because of various characteristics of the metal ions in the bioglass and to enhance its bioactivity and antimicrobial properties. The formation of a hydroxyapatite layer on the surface of the bioactive glass was confirmed through XRD and FTIR analyses.

2. Experimental

2.1. Materials

Precursors of all the chemicals used in the synthesis of bioactive glass were purchased from Sigma Aldrich with 99.99% purity. The chemicals used in the preparation of bioactive glass ceramics are as follows: tetraethyl orthosilicate (TEOS), ethanol (Fischer scientific), ammonia solution (NH₃), calcium nitrate tetrahydrate (Ca(NO₃)₂ · 4H₂O), strontium nitrate (Sr(NO₃)₂) and silver nitrate (AgNO₃). Deionized water was used throughout the synthesis process.

2.2. Sample preparation

To synthesize bioactive glass, we prepared a mixture of TEOS and ethanol using a magnetic stirrer for 30 min at room temperature. A mixture of all the other precursors was prepared separately with distilled water and the mixture of TEOS and ethanol was added drop by drop into this solution under continuous stirring for 1 h. The pH of the final solution was maintained at 11–12 by adding ammonia solution (NH₃) with continuous stirring for 48 h. The final solution was incubated for 48 h to obtain the final gel and the obtained gels were then transferred into the Petri dishes and dried at 80°C for 36 h in a hot air oven. After drying the gels of all the samples were ground into a fine powder using a pestle and mortar into a fine powder and then heated at 600°C to characterize their differences and to conduct various structural studies.

2.3. Characterization

The prepared samples were characterized by X-ray diffraction using a Rigaku Mini-Flex IIX–Ray Diffractometer in the 2θ range of 20–70 degree at a scan rate of 2 degrees/min. The voltage of the generator was 40 kV and a tube current 30mA was recorded. The FTIR spectra measurement to the samples was recorded with a Shimadzu FTIR-8700 in the scanning range from 4000 cm⁻¹ to 600 cm⁻¹. For the FTIR measurement, the powder samples were mixed with KBr and formed into the pellets using a pelletizer. FESEM Supra-55 and HRTEM TECNAI-G20 were used to study the morphology of the glass-ceramics. The particle size and the characteristics of the glass-ceramics were analyzed by HRTEM and selected area electron diffraction (SAED). The bacteria E. coli and S. aureus were used to study the antimicrobial properties of the prepared bioactive glass samples.

2.4. In-vitro study

SBF was synthesized, and the ionic concentrations of the prepared SBF were nearly equivalent to those of human body plasma [26]. This SBF was used for bioactivity testing of our bioglass samples. The prepared samples were soaked in SBF for 7 and 14 days at a temperature of 37°C in an incubation chamber [28,29]. After 7 and 14 days, the immersed samples were taken out of the incubator and cleaned twice with ethanol and then with distilled water. Finally, all the samples were left to dry at room temperature in a desiccator. After drying at room temperature, the samples were analyzed using an X-ray diffraction technique and a field emission scanning electron microscope. The XRD results after the bioactivity testing are shown in Figure 1(b and c). X-ray diffraction showed the growth of a hydroxyapatite layer within 7 days after the immersion in SBF and introduction of strontium into the bioglass matrix hastened the hydroxyapatite growth. The ionic concentrations of human blood plasma and SBF (mmol/L) are given in Table 1 [30,31].

The bioactivity of the bioglass was affected by strontium in two different ways: (a) by influencing the chemical stability and (b) by affecting the structure of the hydroxyapatite formed on the surface of the bioglass. It is well-known that bioactivity of the bioglasses is dependent on the kinetics of the reaction that takes place on the surface when contact is made with SBFs [32,33].
3. Result and discussion

Figure 1(a) shows an X-ray diffraction pattern of the synthesized bioglass powder before the in-vitro study which clearly shows the absence of any crystalline peaks, i.e. the nature of the samples was purely amorphous after they were calcined at 600°C. Samples AgSr(0) and AgSr(3) show the completely amorphous nature of the prepared glass matrix. But sample AgSr(2) shows a low-intensity peak at position 2θ = 38.8° in the XRD pattern which corresponds to \(\beta\)-Sr\(_2\)SiO\(_4\) (JCPDS no # 038–0271) this monoclinic phase is more stable at lower temperatures \([34,35]\). When we reduced the strontium content from 5 mol% to 3 mol% the strontium silicate phase \(\beta\)-Sr\(_2\)SiO\(_4\) was observed we further reduced the strontium content from 3 mol% to 1 mol% in the glass matrix at which point the strontium silicate phase (\(\beta\)-Sr\(_2\)SiO\(_4\)) was completely dissolved in the glass matrix and showed an amorphous nature for the sample AgSr(3).

Figure 1(b, c) shows XRD patterns of the prepared bioglass samples after the in-vitro study, i.e. the bioglass samples on immersed in SBF for 7 and 14 days. It is clear from the X-ray diffraction patterns that many crystalline peaks were present which corresponded to the hydroxyapatite peaks when matched with standard hydroxyapatite (JCPDS # 09–0432). The characteristics major peaks were observed for hydroxyapatite at 2θ = 26°, 32.3°, 34.08°, 39.83° and 49.26°, which shows the presence of crystallinity in the apatite lattice of all the glasses after immersion in SBF for 7 and 14 days \([36]\). The X-ray diffraction results show the changes in the behavior of bioglass after soaking of the samples in SBF for 7 and 14 days, which confirmed the formation of apatite on the surface of the bioglass ceramics. X-ray diffraction peaks observed after the in-vitro study were

| Ion          | Plasma (mmol/L) | SBF  (mmol/L) |
|--------------|-----------------|---------------|
| K\(^+\)      | 5.0             | 5.0           |
| Na\(^+\)     | 142.0           | 142.0         |
| Mg\(^2+\)    | 1.5             | 1.5           |
| Ca\(^2+\)    | 2.5             | 2.5           |
| HCO\(_3^-\)  | 27              | 4.2           |
| HPO\(_4^{2-}\)| 1.0             | 1.0           |
| Cl\(^-\)     | 103.0           | 147.8         |
| SO\(_4^{2-}\)| 0.5             | 0.5           |

Table 1. Human blood plasma and SBF (mmol/L).
exactly equivalent with the peaks of standard hydroxyapatite [36]. This study revealed that, when bioglass samples were immersed in SBF, allowing the bioglass to interact with SBF with an ionic concentrations almost equivalent to human blood plasma, an apatite layer, which is the essential mineral component of bone, developed on the surface of the bioglass [37].

Figure 2 shows the infrared (IR) spectroscopy of the materials in the wavenumber range 4000–600 cm$^{-1}$. In Figure 2(a) the absorption bands observed at 810 and 1097 cm$^{-1}$ are due to vibration of silica units. The band observed at 810 cm$^{-1}$ shows the Si-O-Si symmetric stretching vibration and the band at 1097 cm$^{-1}$ is ascribed to the asymmetric vibration of Si-O-Si units [38,39].

From Figure 2(b) it is observed that FTIR analysis after soaking of the samples in SBF clearly indicates the presence of phosphate peaks in the spectra. The broad peak located at 1060 cm$^{-1}$ and a weak band at 605 cm$^{-1}$ in the transmission spectra show the P-O bending vibrations after soaking in SBF for 7 and 14 days [40,41]. Hydroxyl groups are observed from 3750 cm$^{-1}$ to 3000 cm$^{-1}$. The presence of phosphates after the in-vitro study confirms the formation of a hydroxyapatite layer on the surface of the bioglass ceramics.

Figure 3(a–d) presents the HRTEM images of the prepared bioglass nanoparticles calcined at 600°C for 3 h. The shapes and sizes of the synthesized bioactive glass nanoparticles were determined by high-
resolution transmission electron microscope (HRTEM) analysis. The observed results reveal that silver nanoparticles are embedded in the bioglass matrix and that all the nanoparticles in the prepared glasses are less than 50 nm in size with uniform spherical morphologies. No diffraction rings were observed in the selected area electron diffraction patterns (SAED) which confirms that the prepared glasses are amorphous in nature as a result that is also in good agreement with the XRD.

Figure 4(a, d) shows FESEM images prior to the in-vitro study of the prepared bioglass powders after heat treatment which confirm the formation of spherical agglomerated particles. FESEM analysis was performed on samples calcined at 600°C after different interaction time periods in the SBF to observe the structural morphology of the prepared bioglass samples. Figure 4(b, c, e, f) shows FESEM images of nanoparticles the glass powders after immersion in SBF for 7 and 14 days. It is hard to find

Figure 4. (a, d) FE-SEM images of AgSr(2) and AgSr(3) glass-ceramics before the in-vitro study, (b, c) FESEM images of AgSr(2) glass-ceramics after the in-vitro study for 7 and 14 days and (e, f) of AgSr(3) glass-ceramics after the in-vitro study for 7 and 14 days.

Figure 5. Images of antibacterial experiments on prepared samples for effectiveness against (a) E. coli and (b) S. aureus.
hydroxyapatite (HaP) layer on the surface of the bioglass powders because of the small dimension range involved and the electrical charging. It shows ~100 nm irregularly shaped particles with their sizes are increasing during 7 to 14 days which confirms HaP layer formation on the surface of the bioglass matrix. FESEM images of both samples before SBF immersion exhibited tough surfaces with the primary particles cemented due to incomplete sintering. After soaking for 7 days nanoscale heterogeneous nodules were deposited on the surfaces of the ultrasonically washed discs. With increases in soaking time up to 14 days, a continuous coating layer thought to be an apatite layer covered the surfaces of both samples. XRD and FTIR also confirmed the formation of HaP layers.

### 4. Antibacterial studies

Samples calcined at 600°C for 3 h were used for the antimicrobial study. The antibacterial properties of the samples were examined with respect to gram positive (S. aureus) and gram negative (E. coli) bacteria. The agar disc diffusion method was used to check the antibacterial activity of the prepared bioactive glass. The discs were cleaned with double distilled water two to three times and soaked in a bioactive glass medium overnight for better absorption before being placed on the culture plates. The same experiment was performed in triplicate to assume the accuracy of the results. The culture plates were incubated at 37°C for 24 h. After 24 h of incubation, the zone of inhibition was measured for different samples. It was observed from the antimicrobial results that the silver-free samples had no zone of inhibition with respect to either of the bacteria but that as the silver content increased in the glass matrix, a significant zone of inhibition was seen with respect to both bacteria. The details of the zone of inhibition are given in the Table 2. Consequently, the experiment revealed that the antibacterial effect of the glass matrix can be tuned with very small amounts of silver. In future, therefore, there will be scope to use this glass matrix for various biomedical applications because of its biocompatibility, cost-effectiveness and antibacterial effect.

### 5. Conclusion

We successfully synthesized efficient bioglass ceramics which are bioactive in nature, i.e. capable of bonding with bone through chemical reactions when immersed in SBF. X-Ray diffraction results confirm the formation of a hydroxyapatite layer (HaP) on the surface of the bioglass nanomaterials. The addition of strontium into the present glass matrix allowed the formation of a hydroxyapatite layer after 7 days when the prepared bioglass samples immersed in SBF. The phosphate peaks present in the FTIR spectra confirm hydroxyapatite growth on the surfaces of the bioglass nanomaterials after SBF immersion. The introduction of silver into the prepared glass matrix shows excellent antimicrobial effects and the addition of strontium to the glass composition plays a beneficial role in orthopedic coating and osteoporosis treatment. These materials are especially promising because of its biological effects and its bioactivity in SBF.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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### Table 2. Details of the zones of inhibition of the prepared samples.

| S.No. | Sample code | (E. coli) ZOI (mm) | (S. aureus) ZOI (mm) |
|-------|-------------|--------------------|---------------------|
| 1.    | AgSr (0)    | 0                  | 0                   |
| 2.    | AgSr(2)     | 13.20              | 22.20               |
| 3.    | AgSr (3)    | 26.34              | 22.88               |
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