Bioactive Nanocomposites Composed of Magnesium Oxide Nanoparticles and 58S Bioactive Glass: Synthesis, Characterization, Bioactivity Evaluation and Antibacterial Activity

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Abstract

One of the main strategies to facilitate bone defect healing is the application of a variety of biological materials such as ceramic-based implants and scaffolds. Bioactive glasses are recognized as the favorable biomaterials in bone tissue engineering since they can quickly form a bond with both hard and soft tissues. In this study, novel bioactive nanocomposites based on 58S bioactive glass in composite with various weight percent of magnesium oxide nanoparticles were prepared. DLS measurements represented that 98.1% of 58S glass particles possessed the particle size between 70 and 200 nm. The bioactivity of nanocomposite powders was evaluated through immersion of samples in the simulated body fluid (SBF) at different time intervals of 14 and 28 days. Moreover, the nanocomposite samples were characterized in terms of morphology, phase structure and functional groups using Scanning electron microscopy (SEM), X-ray diffraction and Fourier transform infrared spectroscopy (FTIR) before and after soaking in the SBF solution. It was found that hydroxyapatite was formed on the surface of all nanocomposites after soaking in the SBF solution, although they bioactivity decreased with an increase in the amount of MgO nanoparticles from 15 wt. % to 25 wt. %. Moreover, antibacterial activity of the produced nanocomposites against MRSA bacteria was investigated and the results showed that 58S-15Mg exhibited the highest bactericidal activity.

1. Introduction

For more than half of a century, many studies have been conducted in order to find a reasonable candidate for the reconstruction and repair of the defective bone tissues which were mainly caused by accidental injuries and various bone illnesses such as tumors, trauma or infection (osteomyelitis) [1, 2]. One of the main strategies proposed to eliminate these bone defects has been the application of a variety of biological materials such as ceramic-based implants and scaffolds. Bioceramics are synthetic materials with excellent biocompatibility towards living tissue and therefore can be used in medicine to repair defects and replace damaged tissues [3, 4].

Among the bioceramics, bioactive glasses (BGs) are recognized as the favorable biomaterials in the field of bone tissue engineering since they can quickly form a bond with both hard and soft tissues [5, 6]. The capability of BGs in the formation of a bone bonding was investigated in many researches in which the researchers located the bioactive glasses in contact with the biological media. They have reported that the main reason for the bonding ability was the creation of a hydroxyapatite (HA) layer at the interfacial surface of bioactive glasses and the biological fluids [7–9].

It is known that Prof. Larry Hench was the inventor of bioactive glasses, who synthesized the first ones using the melt-quenching process in 1969 at the University of Florida. Later, his fabricated bioactive glass was named 45S5 Bioglass® due to its composition in weight percent (45% SiO2, 24.5% CaO, 24.5% Na2O and 6% P2O5) [10]. Another technique for the preparation of bioactive glasses was presented in 1991 by Li et al [11]. That named as the sol-gel method. This bottom-up procedure is done at considerably lower
temperatures compared to conventional melt-quenching and constitutes two key reactions of glass precursors namely hydrolysis and condensation [12].

Sol-gel derived inherently porous bioactive glasses can be synthesized in different morphologies among which the nanoparticles have attracted the particular attention of many researchers due to their premium features like great specific surface area and small particle size [13, 14]. In fact, the larger specific surface area of nano-sized bioactive glasses compared to that of micro particles may lead to the enhancement in the hydroxyapatite deposition process and also the formation of tighter bone bonding because more active sites at the interface will be present for osteoblast to attach [15].

It can be found in the literature that some researchers incorporated different metal oxides (MO) like Calcium Oxide, Zinc Oxide and Magnesium Oxide in the structure of SiO$_2$–CaO–P$_2$O$_5$ based bioactive glasses and fabricated BG-MO nano composites to enhance the bioactivity and antibacterial activity of sol-gel derived bioactive glass nanoparticles [16–18]. Due to the significant function of Magnesium element in the human bone metabolism, such as osteoblast differentiation and osteogenic gene expression, [19, 20]. This element based oxides are considered as an appropriate alternative to be applied in the structure of bioactive glasses for the improvement of bioactivity.

It is worth mentioning that magnesium oxide has been incorporated in the preparation of bioactive glasses in different ways. In most of those studies, Magnesium Oxide (MgO) was usually added, as a new component to the composition of common ternary (SiO$_2$–CaO–P$_2$O$_5$) [21–26] or quaternary (SiO$_2$–Na$_2$O-CaO–P$_2$O$_5$) [27–29] bioactive glasses. In these surveys, the researchers used some sources for MgO like Magnesium nitrate hexahydrate in the composition of bioactive glasses and did not use MgO directly as a starting material in sol-gel method. In fact, magnesium oxide was substituted for one of the main constituents (usually calcium oxide) in the formula of bioactive glasses like 58S (58SiO$_2$–33CaO–9P$_2$O$_5$ (wt. %)). For instance, Prabhu et al. [23] decreased the weight percent of CaO and instead added MgO with the weight percent of 10 and 20 to the aforementioned composition of 58S so that the obtained 58SiO$_2$–23CaO–9P$_2$O$_5$–10MgO and 58SiO$_2$–13CaO–9P$_2$O$_5$–20MgO nano bioactive glasses exhibited better in vitro bioactivity compared to common 58S and did not reveal significant antibacterial activity. In another work, [25] the molar composition of CaO was changed by the addition of a different mole percent of MgO in the range of 0–10. In that survey, it was revealed that the bioactivity of synthesized bioactive glasses was firstly increased with an increase in mole percent of MgO and then decreased so that the composition of 60SiO$_2$–4P$_2$O$_5$–31CaO–5MgO (mole %) had the highest formation rate of hydroxyapatite.

In another point of view, some researchers have focused on the direct incorporation of metal oxide nanoparticles into bioglass and fabricated bioactive glass-metal oxide nanocomposites due to the great characteristics of nano bioactive glasses, such as great antibacterial behavior, [30] osteoblast cell adhesion, and proliferation [31]. As an example, Saqaie et al. [32] synthesized bioactive glass-forsterite nanocomposites by adding the various weight percent of forsterite (Mg$_2$SiO$_4$) to the 58S bioactive glass powders and studied its effect on the bioactivity of the prepared samples. They concluded that the
sample containing 20 wt. % of forsterite exhibited the highest bioactivity by the formation of a hydroxyl-carbonate apatite layer (HCA) on the nanocomposite surface.

According to above mentioned explanations, it is clear that different results were reported on the effect of the MgO contents in bioglass composition on the in vitro rate of HA formation [33, 34]. Moreover, to the best of our knowledge, there is no research reporting the effect of direct impregnation of MgO nanoparticles, with a good number of active sites for HA nucleation, into 58S bioactive glass on the bioactivity of resulting nanocomposites. The main goal of this research is to prepare a novel bioactive glass-magnesium oxide nanocomposite by impregnating different weight percent of magnesium oxide nanoparticles into 58S bioactive glass material. Moreover, the effect of MgO nanoparticles content present in the nanocomposites structure on the bioactivity of nanocomposite powders were evaluated through immersion of samples in the simulated body fluid at different time intervals of 14 and 28 days. Finally, the nanocomposite sample with the highest bioactivity was determined by comparing the bioactivity results obtained from characterization tests. Moreover, antibacterial activity of the produced bioactive nanocomposites against Methicillin-resistant Staphylococcus aureus (MRSA) bacteria is investigated.

2. Materials And Methods

2.1. Reagents

The sol-gel precursors used in this study were tetraethyl orthosilicate (TEOS: Si(OC₂H₅)₄), triethyl phosphate (TEP: (C₃H₇)₃PO), calcium nitrate tetrahydrate (CN: Ca(NO₃)₂·4H₂O), ammonia (NH₃), and nitric acid (HNO₃). The molarities of ammonia and nitric acid used in this study were 1M and 2M, respectively. All chemicals were used without further purification and purchased from Merck, Germany.

2.2. Synthesis of 58S nano bioactive glass

In this work, the composition selected for the preparation of bare bioactive glass was 58S [35]. The first step procedure involved mixing TEOS, distilled water, HNO₃ in order. Ethanol as an alcoholic medium was added to the solution and allowed to react for 30 min for the acid hydrolysis of TEOS to proceed almost to completion. The following reagents were added in sequence allowing 20 min for each reagent to react completely: TEP, Ca(NO₃)₂·4H₂O, ammonia solution. After the final addition, mixing was continued until the gel was formed. The gel was kept in the oven and heated at 60 °C for 1 day to remove the residual water and ethanol. Then, the dried sample was calcined for two hours at 600 °C with the heating rate of 3 °C/min to stabilize the glass and eliminate residual nitrate (sample 58S).

2.3. Synthesis of 58S-xMgO bioactive nanocomposites

In order to synthesize bioactive nanocomposites consisting of 58S bioactive glass and magnesium oxide nanoparticles, the sequence of adding precursors was similar to that described in the previous section until the addition of CA (NO₃)₂·4H₂O. Afterward, MgO nanopowders with various weight ratio (MgO to 58S
bioactive glass), of 0.05, 0.15 and 0.25 (5%, 15%, and 25%) were added to the solution. Then, mixing was continued until the gel was formed. Finally, the gel was dried and calcined at the same conditions as mentioned in the previous section. The attained samples are hereafter referred as 58S-xMg samples (Table 1) where x represents weight fraction of MgO nanopowder in the synthesized composite (x = 5, 15, and 25).

| Sample    | Matrix (weight. %) | Reinforcement (weight fraction) |
|-----------|--------------------|---------------------------------|
|           | SiO₂   | CaO   | P₂O₅ | MgO |
| 58S       | 58     | 33    | 9     | 0   |
| 58S-5 Mg  | 58     | 33    | 9     | 0.05|
| 58S-15 Mg | 58     | 33    | 9     | 0.15|
| 58S-25 Mg | 58     | 33    | 9     | 0.25|

### 2.4. Characterization of the samples

A phase structure evaluation was performed by the XRD technique using X-ray diffractometer (XRD, EQUINOX 3000, USA), with CuKα over 2θ range of 10–80° with step size of 0.032° in the fixed time mode, to improve the count statistics. The diffraction patterns were analyzed using the Rietveld structure refinement method as implemented in High Score Plus software. All of the cif files used in this study were identified by the American Mineralogist Crystal Structure Database (AMCSD) codes. The morphological studies of the prepared powders were done using scanning electron microscopy (SEM, EM3200, China) working at 30 kV. Analysis and determination of the functional groups of the samples were performed by Fourier Transform Infrared spectroscopy (FTIR, Bomem, MB-100, USA) using KBr pellets technique in the range of 400–4000 cm⁻¹. Dynamic light scattering (DLS), was done for particle size analysis. DLS is a technique for determining particle size in colloidal suspensions. It often referred to as photon correlation spectroscopy (PCS). In this study DLS test was done by Malvern Zetasizer NANO ZSP ZEN-5600. The laser wavelength, detection angle and laser output are 633 nm (He-Ne laser), 173° and 4.0 mW, respectively. The viscosity of the brine is selected as water to be 0.8872 cP. The refractive indices of dispersant and material are taken to be 1.33 and 1.59, respectively [36].

### 2.5. In-vitro bioactivity analysis

The corrected Kokubo's simulated body fluid (SBF) [37] with ion concentrations similar to that of human blood plasma was applied for in vitro bioactivity assessment of the prepared nanopowders. The samples (58S, 58S-5MgO, 58S-15MgO and 58S-25MgO) with a concentration of 25 mg/ml were immersed in the SBF solution for 14 and 28 days. During the test, the solution was kept at around physiological
temperature in the incubator. At the end of the 14th and 28th days of soaking in the SBF, the solutions were washed with deionized water and then kept at room temperature until dry.

2.6. Antibacterial test

The antibacterial activity of bioactive glasses against MRSA was achieved to investigate the effect of Mg on antibacterial activities in prepared samples. Thus, MRSA was cultured in liquid lysogeny broth (LB) medium at 37 °C and was diluted approximately to $0.5 \times 10^8$ to $2 \times 10^8$ ml$^{-1}$ prior to the experiment. First, 10 mg of each bioactive glass powder and 0.9 ml LB medium were added to 1.5 ml Eppendorf tube followed by stirring for 1 min. Then, 0.1 ml bacterial suspension was added into each Eppendorf tube and the solutions were cultured at 37 °C for 1 h. After a serial dilution, 100 µl suspensions were plated onto LB-agar plates and incubated overnight at 37 °C in the dark.[38] The bactericidal percentages were calculated by counting the final colony-forming units per milliliter (CFU/ml) as follows:

$$\text{Bactericidal fraction} = 1 - \frac{\text{number of survived bacteria}}{\text{number of total bacteria}}$$

2.7. Statistical analysis

Quantitative data were showed as mean ± standard deviation (SD) of at least 3 experiments. The probability value (p) lower than 0.05 was considered as statistically significant.

3. Results And Discussion

In this section, the interpretation of the acquired results is discussed in two main subsections; in the first one, the characterization data obtained from 58S nano bioactive glass before and after immersion in the simulated body fluid are discussed while the second subsection describes those gained for 58S-xMg samples in the same experiments.

3.1. 58S Nano bioactive glass

3.1.1. Surface morphology analysis

Figure 1 shows the SEM images of 58S nano bioactive glass before and after immersion in the SBF for 14 and 28 days. As it is clear from these figures, there was a difference between the morphology of the samples before and after immersion in the SBF. The resulting morphology before immersion indicated the presence of spherical particles. However, it can be seen that new particles have been formed on the 58S bioactive glass after immersion in simulated body fluid, which could be related to the formation of hydroxyapatite on the sample. This change in morphology is in consistency with the results obtained by Taghian et al. [39] who regarded the dissolution-deposition process and the formation of hydroxycarbonate apatite (HCA) as the reason for the morphological change. It is worth mentioning that the presence and formation of hydroxyapatite require further investigation using XRD analysis. According to the SEM images, it can also be concluded that with an increase in the immersion time of the samples in the SBF, the formation of hydroxyapatite clusters was also enhanced.
3.1.2. Particle size analysis

The particle size distribution of 58S bioactive glass based on the number is presented in Fig. 2 using the DLS analysis. A detailed description of DLS results represented that 98.1% of glass particles possessed the particle size between 70 and 200 nm. Moreover, it has been found that more than 80% of the particles were in the range of 70 to 122 nm. The reason for the presence of some particles with larger sizes could be the inadequate dispersion of the synthesized particles in the solution required for DLS measurements. Because of nanobioactive glasses have a higher specific surface area compared to micro-sized, from DLS test, it will expect that the synthesized composites will show good biological properties [40].

3.1.3. Phase structure analysis

The X-ray diffraction pattern of 58S nano bioactive glass powders is observed in Fig. 3. The amorphous or glassy nature of the synthesized 58S sample is confirmed by the resulted broad peaks and the absence of sharp ones. Indeed, the formation of the Si-O-Si network has proven by the broad peaks appeared in the XRD image of the sample in the 2-theta angle range of around 18 to 34 degrees [41]. Moreover, this pattern is in good agreement with the XRD pattern of 58S bioactive glasses prepared by Saravanakumar et al [42].

The X-ray diffraction patterns of 58S nano glass before and after 14 and 28 days of immersion in the SBF solution are presented in Fig. 4. By evaluating the XRD patterns before and after immersion, it is evident that some new peaks were appeared in the XRD image of the sample after immersion compared to that of before immersion. Peaks at approximately 24°, 26°, 30°, 32°, and 39°, [43, 44] angles indicated the presence of the hydroxyapatite phase, on the surface of the sample during in vitro test, according to the card number of 0432-9 based on the Joint Committee on Powder Diffraction Standards (JCPDS). Also, peaks at about angles of 36, 46, 56 and 76 degrees, according to the American Mineralogist Crystal Structure Database (AMCSD) standard number of 0002247, represent the pseudowollastonite phase which has been regarded as a bioactive material [45, 46]. As can be realized from these patterns, increasing the time duration of immersion of 58S nano bioactive glass in the SBF solution, its bioactivity increases concerning the intensity and appearance of hydroxyapatite phase peaks. Accordingly, it can be perceived that the sample immersed in the SBF solution for 28 days, exhibited the highest bioactivity.

3.1.4. Functional groups analysis

The FTIR spectra of the 58S nano bioactive glass powders before and after immersion in the SBF solution for 14 and 28 days are displayed in Fig. 5. The peak observed at the wavenumber of 1090 cm⁻¹ can be related to the asymmetric stretching bond of the Si-O-Si [32]. Obviously, some new peaks have been emerged in the FTIR spectra of 58S nano bioactive glass after exposure to the SBF solution. The appearance of a peak at a wavenumber of about 470 cm⁻¹ in the bioactive glass sample after immersion in the SBF can be attributed to the vibrating phosphate bond (P-O) present in the crystalline apatite layer on the surface of the specimens [39]. Besides, the peak at 1090 cm⁻¹ became sharper, which can be imputed to the destruction of the Si-O-Si network to form hydroxyapatite. This reasoning can be understood from the available typical absorption band of hydroxyapatite seen in the wavenumber area of
910–1040 cm$^{-1}$ attributed to PO$_4$ [47]. Also, peaks corresponding to the wavenumbers of 603 and 565 cm$^{-1}$ represent the P-O bond in PO$_4$ in the apatite network [39]. The peak at 800 cm$^{-1}$ is related to the symmetric stretching vibration of Si-O-Si [48]. Two other visible peaks at the wavenumbers of 1651 and 3500 cm$^{-1}$ can be ascribed to the tensile vibrations of O-H in the Si-OH groups due to the absorption of water in the solution. This indicates that the glass surface possessed a large number of silanol groups [49]. The presence of water may be due to the presence of strong nucleophilic groups such as P-OH or Ca-OH, which has led to moisture absorption [50]. The peak occurred at 1503 cm$^{-1}$ is related to carbonate bonds in hydroxyapatite [51]. Also, the peak at the wavenumber of about 1455 cm$^{-1}$ is related to the C-O bond in the carbonate groups substituted for phosphate groups in the apatite network [52]. It should be noted that these results are verified by the findings reported by Moghanian et al [38]. Regarding the intensity of the peaks corresponding to the hydroxyapatite phase, it can be concluded that the 58S sample with the highest bioactivity was obtained after 28 days of soaking in the SBF.

3.2. 58S/x-Mg nanocomposites

In this section, the characterization data of the prepared nanocomposites, containing 58S bioactive glass and different amounts of magnesium oxide nanoparticles, obtained before and after immersion in the SBF were demonstrated and also compared with those characterization data represented in the previous section for 58S nano bioactive glass.

3.2.1. Surface morphology analysis

Figure 6 shows the SEM images of the 58S-25Mg nanocomposite before and after soaking in the simulated body fluid for 14 and 28 days. As displayed in Figure 6a, the synthesized nanocomposite possessed spherical nano particles. Furthermore, based on the pictures taken from this sample after 14 (Figure 6b) and 28 days (Figure 6c) of soaking in the SBF solution, it is observed that hydroxyapatite clusters were formed on the sample. It can be proven that bioactive glass with spherical particles exhibits superior biological activity due to regular shape [40]. Also, it can be seen that more clusters were present on the surface of the sample after 28-day immersion. The reason for this phenomenon can be this matter that as the days of soaking increased from 14 to 28, the nuclei created by apatite were grown as well and then distributed throughout the sample so that they covered almost an array of sites on the surface of the prepared nanocomposite [38].

3.2.2. Phase structure analysis

The x-ray diffraction patterns of 58S-5 Mg, 58S-15 Mg and 58S-25 Mg bioactive nanocomposites are illustrated in Fig. 7. The peaks appeared in this figure at the approximate angles of 42, 62, 75 and 79 degrees are assigned to magnesium oxide nanoparticles, according to Fig. 8 in which the XRD pattern of purchasing MgO, presented by its manufacturer, is displayed. The XRD analysis of nanocomposites containing 58S bioactive glass and magnesium oxide nanoparticles shows that the synthesized samples exhibited glassy and amorphous structure, whereas the peaks confirming the presence of magnesium
oxide were also noticeable. In addition, it is worth mentioning that the glassy and amorphous characteristics of these nanocomposites were not varied with an increase in the weight percent of magnesium oxide nanoparticles. On the other hand, it can be concluded that as the amount of magnesium oxide incorporated in the 58S-xMg nanocomposites increased, the intensity of the magnesium oxide's peaks enhanced, too.

To evaluate the bioactivity of prepared nanocomposites, the X-ray diffraction patterns of the 58S-25 Mg sample before and after 14 (58S-25 Mg-14d) and 28 (58S-25 Mg-28d) days of immersion in the SBF solution are displayed in Fig. 9. The peaks corresponding to magnesium oxide and hydroxyapatite were specified in the patterns. According to the intensity of the hydroxyapatite peaks, it can be understood that 58S-25 Mg bioactive nanocomposite showed more bioactivity after 28 days of immersion in comparison with 14 days of immersion. The XRD pattern of the (58S-25 Mg-14d sample demonstrates that the hydroxyapatite peaks were formed beside the magnesium oxide peaks, although their intensity was lower than the intensity of magnesium oxide peaks.

It's worth noting that this is not because the intensity of magnesium oxide peaks present in the 58S-25 Mg XRD pattern was changed after immersion in the SBF solution, but it also confirms that HA peaks emerged with approximately the same intensity as the existed MgO peaks. This issue can be observed in the XRD spectrum of the 58S-25 Mg sample after 28 days of immersion in the SBF solution, in which the intensity of the hydroxyapatite peaks (e.g., at 32°) increased to a level that the magnesium oxide peaks became less prominent in the pattern. So, based on these results, it is clear that the highest bioactivity was obtained for the 58S-25 Mg-28d sample since the peak of apatite diffraction became sharp and intense with a rise during the soaking time [38].

Figure 10 illustrates the X-ray diffraction patterns of the 58S, 58S-5Mg, 58S-15Mg, and 58S-25Mg samples after 28 days of immersion in the simulated body fluid for the investigation of the MgO effect on the bioactivity of sol-gel derived bioactive glasses. According to the intensity of hydroxyapatite peaks present in these XRD spectra, it is found that as the amount of magnesium oxide nanoparticles increased from 5 to 15 wt. %, the bioactivity of the nanocomposites increased; however, as the amount of magnesium oxide incorporated in the nanocomposite increased from 15 wt. % to 25 wt. %, the lower bioactive sample were obtained at the end of 28-day soaking. In other words, the 58S-15Mg bioactive nanocomposite has higher bioactivity than the other two nanocomposites.

A survey of the previous studies carried out in this area indicates that similar results were acquired using a bioactive glass based on the CaO-MgO-P$_2$O$_5$-SiO$_2$ system which represented less bioactivity with an increase in the magnesium content from 5 to 20 mole% substituted for CaO [53]. In another study, they also concluded that the apatite phase peaks were not observed for the glass sample with the highest MgO content (20% MgO). On the other hand, referring to these XRD patterns in this study, it is shown that even the nanocomposite prepared using 58S bioactive glass and the highest content of MgO nanoparticles (58S-25 Mg) showed the formation of hydroxyapatite on the nanocomposite surface [24].

3.2.3. Functional groups analysis
A comparison of the FTIR spectra of 58S-5MgO, 58S-15 Mg and 58S-25 Mg samples before immersion in the SBF is presented in Fig. 11. As mentioned earlier, the peak at the wavenumber of 1090 cm$^{-1}$ is related to asymmetric stretching bond Si-O-Si [41]. The main absorption band obtained at 1250 cm$^{-1}$ can be assigned to the asymmetric stretching of Si-O-Si [25]. On the other hand, the peaks in the wavenumber range between 440 and 900 cm$^{-1}$ may be related to the stretching bond of Mg-O [54–56].

Figure 12 shows the FTIR spectrum of the 58S-25Mg sample before and after immersion in the SBF for 14 and 28 days. The main peaks seen in the XRD pattern of the 58S-25Mg sample before immersion are described in Figure 11. In this section, the comparison of the peaks observed in the FTIR spectra of 58S-25Mg before and after soaking in the SBF solution is discussed. Two new peaks are observed in the FTIR spectra of 58S-25Mg after soaking at about 1630 and 3440 cm$^{-1}$ which can be ascribed to the O-H bond due to the water presence. This may be due to the presence of groups such as P-OH or Ca-OH, which result in moisture absorption [50]. The peaks appeared in the range of 1400 to 1500 cm$^{-1}$ are related to the C-O bond, which represents the formation of hydroxycarbonate apatite (HCA) layer on the surface of nanocomposite samples [53]. Another new peak, which is observed at the wavenumber of 603 cm$^{-1}$ indicates the presence of the PO$_4$ bond in the apatite network [39]. Also, the peak at the wavenumber of about 870 cm$^{-1}$ is attributed to the C-O bond in the carbonate groups in phosphate groups in the apatite network [52, 57]. As can be seen, the peak at 1090 cm$^{-1}$ became sharper after immersion in the SBF, which could be due to the destruction of the Si-O-Si network to form hydroxyapatite. Therefore, it can be concluded that as the intensity of this peak enhanced, more hydroxyapatite was formed on the nanocomposite surface [47].

Figure 13 shows the FTIR spectra of 58S, 58S-5Mg, 58S-15Mg and 58S-25Mg samples after immersion in the SBF for 28 days. The comparison of these FTIR patterns is a good way to evaluate the bioactivity of nanocomposites containing various weight percent of magnesium. The specified peaks in this figure were described in the previous sections. As mentioned before, the formation of the crystalline apatite layer on the surface of the nanocomposite samples is verified due to the P-O vibrating bonds appeared at the wavenumbers of 566 cm$^{-1}$ and 607 cm$^{-1}$ [39]. Also, Peaks observed around wavenumber of 1392-1435 cm$^{-1}$ range, assure the presence of carbonate group into the apatite layer [58].

From the above description, it can be concluded that 58S bioactive glass exhibited high bioactive property compared to other samples (58S-5 Mg, 58S-15 Mg, and 58S-25 Mg) at end of 28 day soaking in SBF solution. It can be attributed to the different porosity of the 58S bioactive glass and synthesized nanocomposites. Higher porosity supplies more sites for precipitation. Another reason for this result can be attributed to many factors like, the speed and manner of the migration of Ca$^{2+}$ and PO$_4^{3-}$ groups to the surface forming CaO\PO$_4^{3-}$ clusters on the top of the SiO$_2$-rich layer was done, followed by growth of the amorphous CaP. After that, the crystallization of the amorphous CaP [59]. Also, it can be concluded that 58S-15 Mg sample has exhibited higher bioactivity in comparison to the other nanocomposite of BGs, by investigating both, FTIR spectra and XRD patterns of the samples. A similar consequence has been concluded by Moghanian et al. they reported that presence of magnesium in 58S bioactive glass
increased bioactivity of BGs first, after that by adding more magnesium to 58S bioactive glass, the bioactivity of sample decreased [25].

3.2.4. Antibacterial test

Figure 14 presents the comparative study of the antibacterial activity of synthesized bioactive nanocomposites against MRSA bacteria. According to the obtained values for bactericidal activity, 58S-15Mg possessed higher bactericidal efficiency against MRSA bacteria than 58S-5Mg and 58S-25Mg while showing lower bactericidal efficiency compared to 58S nano bioactive glass. These results revealed that MgO exhibited remarkable dose-dependent antibacterial activity against MRSA bacteria, i.e. by increasing the amount of MgO substituted in bioactive nanocomposite from 5 to 15 wt. %, its bactericidal efficiency significantly increased. However, the sample with the 25 weight percent MgO had a reverse effect and led to a significant decrease in the bactericidal efficiency than the 58S-15Mg. It must be noted that, many factors are related to antibacterial activities of bioactive glass (like the presence of ions such as phosphate, calcium, and magnesium), but the precise mechanism of it is not discovered yet [60]. In brief, for present study among the synthesized nanocomposites, the 58S-15Mg sample exhibited the highest antibacterial activity of 37.5%.

4. Conclusions

Novel bioactive glass-magnesium oxide nanocomposites by adding different weight percent of magnesium oxide nanoparticles to 58S bioactive glass material were successfully synthesized by the sol-gel method. Then, the effect of MgO nanoparticles content present in the nanocomposites was investigated on the bioactivity of nanocomposite powders through the immersion of the samples in the simulated body fluid (SBF) at different time intervals of 14 and 28 days. Moreover, the antibacterial activity of the produced nanocomposites against MRSA bacteria was studied. The results are as follows:

1. Amorphous and glassy nature of the synthesized samples (58S, 58S-5 Mg, 58S-15 Mg and 58S-25 Mg) was verified by their XRD patterns.
2. According to the XRD patterns and the FTIR spectra of the samples after soaking in the SBF solution, 58S, 58S-5 Mg, 58S-15 Mg and 58S-25 Mg were bioactive materials due to the formation of hydroxyapatite on their surface.
3. XRD results also confirmed that the bioactivity of nanocomposites first increased with an increase in the content of magnesium oxide nanoparticles up to 15 wt. % and then decreased as the weight percent of MgO nanoparticles changed from 15 wt. % to 25 wt. %.
4. The antibacterial activity assessment showed that 58S-15 Mg had more antibacterial activity than two other nanocomposites containing magnesium oxide nanoparticles.

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**Figures**
Figure 1

SEM images of 58S bioactive glass (a) before, (b) after 14 days and (c) after 28 days of immersion in the SBF.

Figure 2

The DLS analysis of 58S nano bioactive glass.
Figure 3

XRD pattern of 58S nano bioactive glass.
Figure 4

XRD patterns of 58S nano bioactive glass before (58S sample), after 14 days (58S-14d sample) and 28 days (58S-28d sample) of immersion in the SBF solution.
Figure 5

FTIR spectra of 58S nano bioactive glass before (58S sample), after 14 days (58S-14d sample) and 28 days (58S-28d sample) of immersion in SBF solution.
Figure 6
SEM images of 58S-25Mg bioactive nanocomposite (a) before, (b) after 14 days and (c) after 28 days of immersion in the SBF.

Figure 7

XRD patterns of 58S-5Mg, 58S-15Mg and 58S-25Mg nanocomposites.
Figure 8

XRD pattern of used MgO represented by its manufacturer.
Figure 9

XRD patterns of 58S-25Mg bioactive glass nanocomposites, after 14 and 28 days of immersion in the SBF solution.
Figure 10

XRD pattern of 58S, 58S-5Mg, 58S-15Mg, and 58S-25Mg samples after 28 days of immersion in the SBF.
Figure 11

FTIR spectra of 58S-5Mg, 58S-15Mg and 58S-25Mg samples before immersion in the SBF.
Figure 12

FTIR spectra of 58S-25Mg bioactive glass nanocomposite after 14 and 28 days of immersion in the SBF solution.
Figure 13

FTIR spectra of 58S, 58S-5Mg, 58S-15Mg, and 58S-25Mg bioactive glass nanocomposites after 28 days of immersion in the SBF.
Figure 14

Antibacterial activity of 58S, 58S-5Mg, 58S-15Mg, and 58S-25Mg bioactive glass nanocomposites