

RESEARCH ARTICLE

Potential of magnetite nanoparticles with biopolymers loaded with gentamicin drug for bone cancer treatment

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ABSTRACT

Objective(s): Due to the natural bone microstructure, the design and fabrication of porous ceramic scaffold nanocomposite materials coated with a thin layer of a natural polymer can provide an ideal scaffold for bone tissue engineering. This study aimed to fabricate multi-component porous magnetic scaffolds by freeze-drying (FD) technique using a gelatin polymer layer coated with a gentamicin drug.

Methods: Magnetic nanoparticles (MNPs) can be manipulated and controlled by an external magnetic field gradient (EMFG) that is inherent in the magnetic field's permeability within human tissues. In the present work, unlike the usual ceramic/polymer composite scaffold, the ceramic components and the magnet were placed together in the reaction medium from the beginning, and bioceramics were replaced in the composite polymer network and then coated with a drug-loaded polymer. To evaluate the morphology of the magnetic scaffold, scanning electron microscopy (SEM) was utilized to evaluate the microstructure and observe the porosity of the porous tissue.

Results: After analyzing the SEM images, the porosity of the scaffolds was measured, which was similar to the normal bone architecture. The addition of gentamicin to the gelation was investigated to monitor the drug delivery reaction in the biological environment. The magnetic properties of the sample were evaluated using the hyperthermia test for 15 seconds at the adiabatic conditions. Also, the porosity value increased from 55% to 78% with the addition of MNPs to the based matrix.

Conclusions: The results of this study showed that gentamicin-gelatin-coated on porous ceramic-magnet composite scaffolds could be used in bone tissue engineering and apply for treatment of bone tumors, because of their similarity to the bone structure with good porosity.

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INTRODUCTION

Magnetic nanoparticles (MNPs) can be manipulated and controlled by the external magnetic field gradient (EMFG), which is inherent in the magnetic field permeability within human

bone tissues [1-4]. This function, remote control, and monitoring ability were used in the transfer and accumulation of MNPs, the specific labeling of biological microstructures, and in particular, in the anticancer drug delivery to the target tumor tissue [5-7]. The application of bioactive ceramics

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with these MNPs caused chemical interactions with the surrounding bone. Each tissue of the body itself has a hierarchical microstructure and consists of several surfaces starting from the macroscopic comparison (centimeter range) and continuing to the molecular level (nanometer range) [8-10]. The smallest surface from which the underlying tissue function derives is called the functional subunit, which is usually about 100 microns in scale, while different cell measure is about 10 microns [11-18]. Therefore, cells are located in a microenvironment where tissue function was not observed on a smaller size. The bones and cell tumors were damaged and invaded by inserting into the magnetic field. MNPs and these new techniques could be used to treat and eliminate these cancer cells and bone tumors [19-22]. In medical applications, there are generally two forms of superparamagnetic: magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) nanoparticles. These magnetite particle sizes are often in the range of 5–30 (nm) and have a size distribution of about 10–20%. Solubility in water is essential for the use of these nanoparticles in biomedical approaches. By replacing the iron in the magnetite and maghemite microstructures with cobalt and nickel, the magnetic properties changed to the various magnetically responses [23-26].

Cancer has the most important rank in the leading causes of death in all countries after heart disease and accident ratio. The leading cause of this disease is a mutation in the body's cells, and the mutant cells multiply at a higher degree than healthy cells, leaving nutrients and oxygen out of the cells. Many medications have been studied to solve this problem [19, 21]. Hyperthermia is a new treatment based on the mechanism of heat, and magnetic field on the tumor site. This type of cancer treatment is complementary to treatments such as chemotherapy, radiation therapy, surgical treatment, gene therapy, and immunotherapy in modern and developed countries. Hyperthermia has been applied to treat bone and soft cancer with controlled clinical trials [22-28]. During these experiments, cancer cells were found to be more susceptible to hyperthermia than normal cells.

Unlike healthy tissues that tolerate 42-46°C, cancer cells in this temperature range develop apoptosis (programmed death of the cell). Treatment and heat therapy with temperatures above 46 degrees is called thermal erosion. In thermal ablation, the tumor temperature is raised to 46°C (up to 56°C), leading to cell necrosis, clotting,

or charcoal. This action can lead to necrosis of healthy cells in the body and surrounding tissues. Therefore, thermal ablation is not desirable, and hyperthermia induces apoptosis [29-33]. In mild hyperthermia, there are various effects on both cellular and tissue levels. The cells were subjected to heat stress, which resulted in the activation or inhibition of many of the cellular and intracellular degradation mechanisms such as protein folding, density, and cross-linking between DNA. Cells are more sensitive to heat at the mitosis stage, in which cancer cells are more sensitive to heat than healthy cells at the cell division stage. On the other hand, the selective effects of hyperthermia on tumors are due to the physiological differences between them and healthy tissues. Compared to healthy tissues, vascular distribution in tumors is abnormal [34-36]. In many cases, the tumor blood flow is higher than the surrounding healthy tissue, especially in smaller tumors, as the tumor blood flow generally decreases with increasing size. These environmental factors in the tumor make cancer cells more susceptible to hyperthermia. There are various heat sources for hyperthermia in clinical applications [37-41]. The major drawback of using these MNPs in medical applications is their high sensitivity to oxidation, which uses coatings or alloys such as platinum, cobalt, and carbon to solve the problem. Among the magnetic materials, iron oxide nanoparticles are the only magnetic materials that have suitable properties for use in biomedicine [42-46]. Hence, these parameters are particularly important for biomedical diagnostics applications such as nanoparticles that can be used to enhance the contrast of magnetic resonance images (MRI) [47-48]. In this study, freeze drying method was used to fabricate three-dimensional (3D) porous polymeric-ceramic scaffolds using gentamicin-loaded drug in gelatin polymer. The following magnetically, and biological properties were tested to select the best and appropriate scaffold.

MATERIALS AND METHODS

Methods for the synthesis of magnetic nanoparticles (MNPs) in the liquid phase include coprecipitation, micro emulsion, and ultrasound are known as the best technique to produce this biomedical nanoparticle [9]. Co-precipitation is the most straightforward and most efficient chemical technique for the synthesis of metal oxides and ferrite nanoparticles. In the current work, a specific amount of gelatin was dissolved in

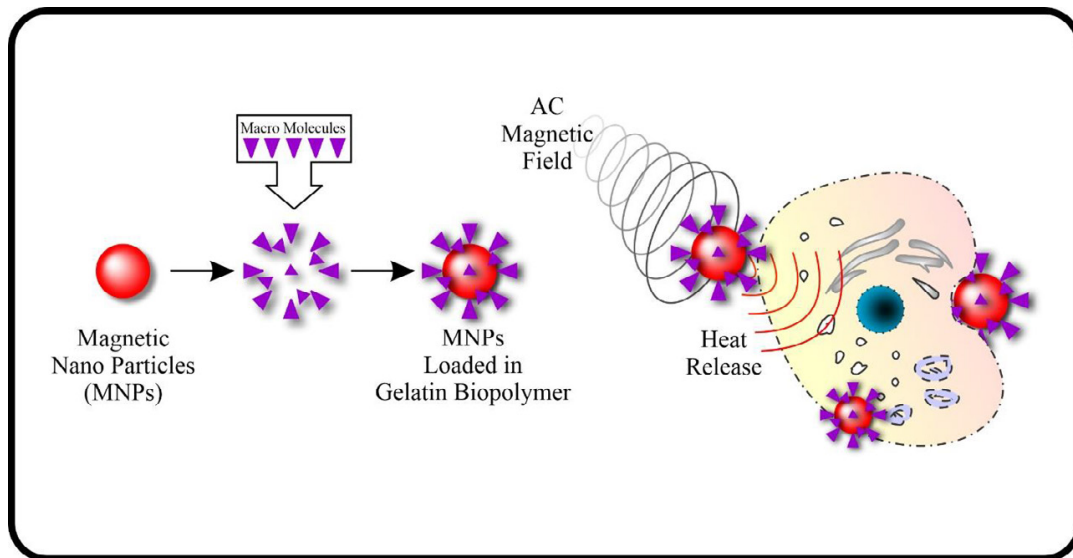


Fig. 1. Schematic of application of magnetite nanoparticles using for hyperthermia term

the distilled water containing 2 vol. % acetic acid in a slurry balloon equipped with a continuous stirrer with a nitrogen gas inlet at 60°C. Then, potassium persulfate was added to the gelatin biopolymer solution as an initiator. After 30 min, various contents of MNPs powder were added to the based biopolymer solution, which stirred for hours. Finally, the polymerization was stopped by cooling the solution and removing the nitrogen gas stream. After the emulsion was removed from the freezer, the samples were frozen entirely in freeze-drying tools. The fabrication of porous scaffolds was carried out in the freeze-drying technique (DOORSATECH company, Iran made) apparatus for 24 hours at -45°C and 0.5 millibars. After fabrication of the specimens, the mechanical properties of the dry sample, such as tensile strength, the elastic modulus was measured experimentally. Fig. 1 shows the schematic image of MNPs used in the cancer treatment approaches in the AC magnetic field. As can be seen in Fig. 1, magnetic nanoparticles enter the macromolecules loaded in Gelatin biopolymer. Schematic of the work explained that the MNPs under AC magnetic field can absorb heat and then release heat beside the tumor to change the tumor size. The sample was performed for 10 seconds to monitor temperature changes at the AC magnetic field from an electric solenoid tube in the presence of ethanol. Also, a Vibrating Sample Magnetometer (VSM) analysis was conducted to measure the magnetic

behavior of the scaffold and the hyperthermia evaluation of the targeted cell. Also, the following schematic represents the drug release rate and the heat transfer rate in ceramic nanocomposites with different weight percentages.

MATERIALS CHARACTERIZATION

X-ray diffraction (XRD) analysis and phase determination

The X-ray diffraction (XRD) and Equinox 300 tests were performed in the range of 2θ between 10 and 80 degrees below 40 kV and 30 mA to determine the phases in the synthesized powder and the composite scaffold.

Morphology evaluation by scanning electron microscope (SEM) analysis

A scanning electron microscope (SEM) and Vega II machine made in the CZECH Republic under 15-20 kV were used to consider the morphology of the magnetic scaffold surface. A thin layer of gold was sprayed on the specimens before photographing to enhance the surface electrical conductivity of the specimen, which leads to greater clarity of the images. In this work, the sample was soaked in the Simulated Body Fluid (SBF) for 28 days at 37°C to consider its degradation and apatite formation rate while it can influence other mechanical and magnetically behavior results. The hyperthermia evaluation of the sample was performed for 10 seconds to monitor temperature changes at the

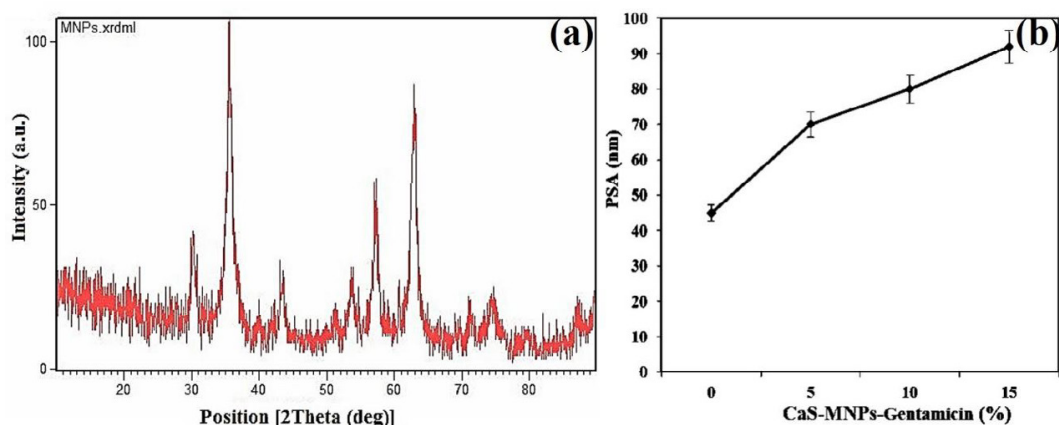


Fig. 2. (a) XRD pattern of MNPs, and (b) PSA results of the composite Nano powder

AC magnetic field and electrical solenoid tube in the presence of ethanol. Also, a VSM analysis was conducted to measure the magnetic behavior of the scaffold.

RESULTS

In this section, after the fabrication of ceramic-magnet nanocomposite scaffolds with various amounts of MNPs loaded with gentamicin drug, morphological analysis was performed. Using SEM images to monitor porosity size and distribution. The gelatin biopolymer was distilled in 50 mL deionized water and stirred for several hours at 50°C. Then, 1 mg gentamicin drug without perseverative composed with the homogenized gelatin solution. Fig. 2 (a) shows the XRD pattern of magnetic ceramic used in the preparation process of scaffolds. The XRD pattern describes the application of magnetic nanoparticles for cancer cell treatment in which the size of nanoparticle is important and can affect the magnetic results. The XRD pattern shows that after the MNPs being inserted into the magnetic field, it causes damage to the cancer cells at temperatures above 38°C. The XRD analysis was used to examine the crystallography of nanoparticles and bio-nanocomposites. Fig. 2 (a) shows the XRD pattern of standard magnetic nanoparticles that have a completely crystalline structure. The VSM results analysis of Fe_3O_4 nanomaterials is shown in Fig. 5(a). As shown in the diagram of Fe_3O_4 nanoparticles, the magnetic property of these

nanoparticles is about 55.20 emu/g at 4000 Oe, which indicates the superparamagnetic properties of Fe_3O_4 nanoparticles and also shows that Fe_3O_4 nanoparticles are suitable for the synthesis of bio-nanocomposites.

As noted earlier, hypoxic areas become deficient, and acidosis occurs due to decreased blood flow due to increased heat. These environmental factors in the tumor make cancer cells more susceptible to hyperthermia. These peaks are related to the crystalline region of the magnetite nanoparticles. Also, sharp peaks, with low width, indicating that the phase is crystalline and high in crystallinity (Table 2). The most significant peaks in the XRD pattern shown correspond to 2θ of 36 and 63°. Fig. 2 (b) shows the particle size analysis of the nanocomposite using PSA tools, which shows that the addition of MNPs increases the particle size of the matrix, indicating a particle distribution of about 40-100 nm. Fig. 3 (a) shows the comparison between the porosity percentage of the nanocomposite, which shows the increase in porosity of the scaffold by the addition of MNPs contents (see Table 1). Also, this Figure shows that with the addition of MNPs, the gentamicin release rates are increasing, which can be due to its various porosity or wettability properties of the scaffold in the physiological saline. Fig. 3 (b) shows that as the MNPs content increases, the rate of apatite or bone growth in the SBF increases. The results showed that the sample containing 10 wt% and 15 wt% MNPs has a constant dissolution rate with the same

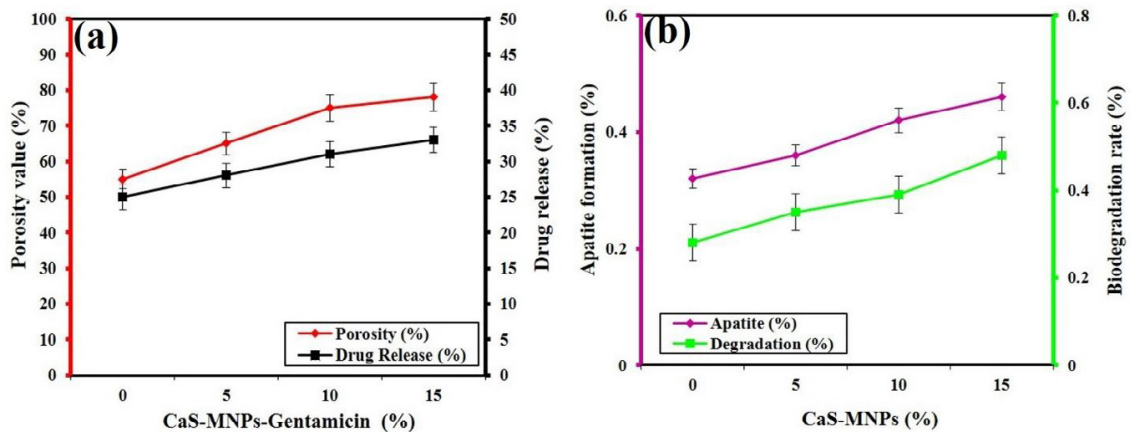


Fig. 3. (a) Porosity chart results comparing with quantifying drug release in porous scaffolds containing gentamicin, and (b) diagram of apatite examination and degradation rate of the prepared samples

Table 1. The mechanical and biological result of prepared bioceramic-MNPs- gelatin-gentamicin prepared using freeze drying technique

Material Properties	Poisson's ratio	Porosity (%)
0	0.30	55
5	0.30	65
10	0.32	75
15	0.34	78

Table 2. Crystalline size of the powder composed with MNPs

Sample	Composite crystalline Size (nm)
0 %	55
5 %	65
10 %	75
15 %	78

trend as the sample with 5 wt% represented. The apatite formation of the porous scaffold improved with the addition of MNPs to the CAS from 0.32 to 0.46 measures using SEM images. The results of immersion of the samples in the phosphate buffer saline (PBS) showed that the sample with the highest amount of MNPs had the highest weight loss. The high viscosity of the solution and the high density of the magnet made it lose weight and allow the ions to enter the solution faster. Examination of the sample results without MNPs content revealed that the samples were almost completely dissolved due to insufficient strength and calcium ion. Silicon ion contributed to the deposition of

apatite on the surface of the specimens, which changes in the concentration of silicon ions were solely due to the dissolution of MNPs and calcium silicate ions. The following changes with an increasing percentage of MNPs in the porous scaffold, indicating a lower dissolution rate of the samples with higher MNPs percentages. As time passed, the calcium and magnetite ions became larger, resulting in a more significant difference and greater absorption in the solution. Fig. 4 shows the SEM images of well-porous scaffolds. As can be seen, the pores are interconnected and help to interfere with the drug by interconnecting with each other. The walls of these pores are composed

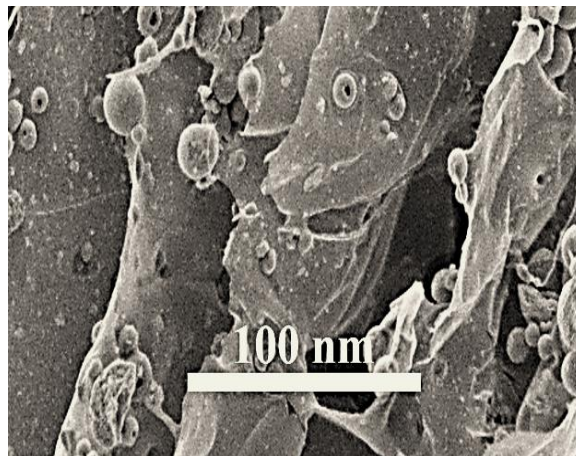


Fig. 4. SEM micrograph of the 15 wt% MNPs in the porous bio-nanocomposite loaded with gentamicin prepared using freeze drying technique

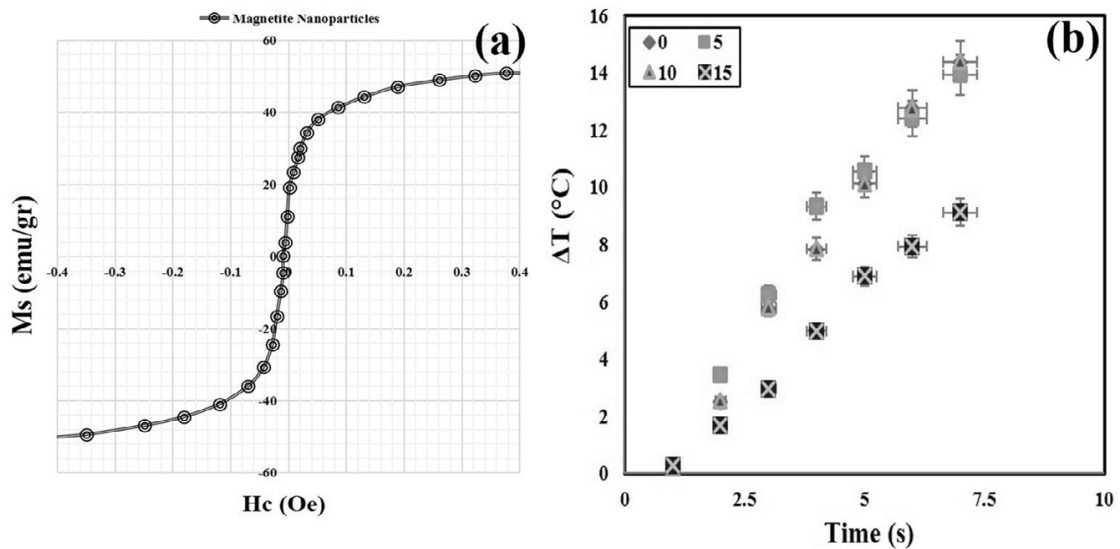


Fig. 5. (a) Investigation of magnetism of MNPs, and (b) diagram of the temperature changes of the freeze-dried scaffolds under the influence of the alternating current magnetic field

of gelatin-magnet/bioceramic nanocomposites. The amount of bioceramic nanoparticles directly affects the morphology of the scaffold. According to SEM images, the scaffolds consist of randomly sized pores that are interconnected. The porous microstructure of these nanocomposites provides a suitable environment for cell growth and material loading purposes. It is easy to locate cells and growth factors, encapsulate drug molecules, enter nutrients, and release oxygen through these three-dimensional bio-nanocomposite scaffolds. In terms of apatite growth and development for

tissue engineering applications, the pore size of the scaffolds must be several times larger than the size of the deposited cells. Fig. 5 (a) examines magnetic nanoparticles in a solid phase which responds to magnetism. The VSM analysis can be either single nanoparticles or aggregates of micro and nanoparticles [18-22]. The composition, size, and the synthesis route of magnetic nanoparticles vary according to their type of application, but superparamagnetic and ferromagnetic particles are applicable for a variety of drug applications. Such materials are strongly affected by the external

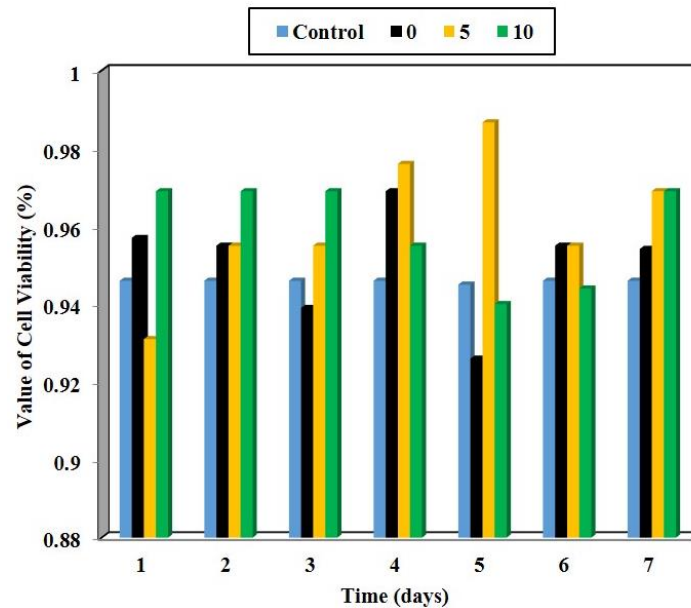


Fig. 6. Cell viability of the porous scaffold coated using gentamicin after 7 days of incubation

magnetic field due to the lattice unit's magnetic moment and the microstructure of the domains. Therefore, the MNPs act as an inactive particle in the absence of the external magnetic field [6-9]. The single-domain and super-paramagnetic properties of magnetic nanoparticles are the sources of their unique features. Fig. 5 (a) represents the results of magnetic and, calorimetry results of the ceramic-magnetic nanocomposite scaffold showed heat generation under the influence of alternating magnetic (AM) field. The produced-heat can be overbearing for painful bone cancer cells and cause them to die over time. Microscopic magnetic implants are currently being used around and in the tumor area to treat cancer cells. Also, using magnetic treatment and hyperthermia in the clinical phase for most of the cancers, most efforts are on the potential of the magnetic nanoparticles that are applicable to generate sufficient heat. In the current study, Fig. 5(b) shows the temperature changes in terms of time changes for four samples with various content of MNPs in the scaffold with the adiabatic condition. As the intensity of the applied magnetic field increases, the acceleration of the magnetic field decreases to reach its saturation. As can be seen, with increasing time from 0 to 10 seconds, the temperature of all samples increased from 0°C to 14°C, with the sample having a higher temperature by 10 wt% than the other samples. The

treatment and the amount of hyperthermia method depends on the temperature applied during the treatment and the characteristics of the damaged tissue and the invaded cell. To ensure proper and balanced temperature, the temperature of the tumor and surrounding tissue should be monitored during hyperthermia treatment. In magnetic hyperthermia, magnetic nanoparticles generate heat under alternating magnetic field radiation under a process called magnetic displacement. One of the things that cause sufficient temperature distribution in all parts of the tumor and does not damage healthy cells during the process is accurate and excellent targeting of nanoparticles with appropriate targeting agents.

DISCUSSION

Sahmani et al [15] produced a novel porous scaffold made of hydroxyapatite composed with pure magnetite nanoparticles using a space holder technique coated with ibuprofen drug for bone cancer application. The obtained results indicated that the addition of magnetite nanoparticles affects the compression strength and porosity significantly. In another work, Monshi et al. [17] used a fused deposition modeling technique and electroconductive filament for the preparation of complex scaffold for bone tissue approaches. They prepared a scaffold with a various topology

such as cubic and cylindrical and consider its static load-bearing in ABAQUS software. Kordjamshidi et al. [21] fabricate a porous polymeric scaffold using calcium silicate nanoparticle using a freeze-drying technique loaded with celecoxib drug bone for regeneration containing various amounts of magnetite nanoparticles. A comparison of the results of the current work with other research studies in the magnetic scaffold represented that the porosity and the degradation rate of the fabricated scaffold changed the magnetic behavior influenced by its porosity ratio [49-51]. Evaluation of the toxicity of porous scaffolds made of gentamicin coated on a ceramic scaffold was determined under stromal cells placed in a 6-well plate containing 100,000 stromal cells in the MTT assay. The porous scaffolds were then kept in the incubator for 7 days by maintaining standard conditions in terms of temperature and humidity assessment. In the next step, to evaluate the cytotoxicity of the obtained scaffolds was determined using ELISA Reader at 530 and 630 wavelengths. The results showed that the rate of survival and cellularity in scaffolds containing a higher percentage of MNPs was observed in the first few days, while in the last two days the cells suffered significantly more cell death than the control group, but due to the presence of drug functional groups, no significant changes were seen among the scaffolds made according to as shown in Fig. 6.

Also, the functional groups release into the solution that may accelerate the apatite formation of the surface can also increase the magnetic response regarding the absorption of negatively charged ions like silica ions. Then, gentamicin with the chemical formula $C_{21}H_{43}N_5O_7$ increased the magnetization behavior and initiated the release process until reaching an almost constant value. These changes indicated the deposition of calcium compounds over time. As time passed due to the simultaneous dissolution of calcium ions, the concentration of calcium ions reached equilibrium and relatively stable. However, since silicate bioceramics have a wide range of chemical constituents, their physical and chemical properties, as well as their biological properties, can be optimized to meet the requirements for optimal restoration with a positive effect on the release rate of the antibiotic and the beneficial effect on the bacterium. The disadvantages of modern calcium silicates are their rapid degradation and dissolution, which can be controlled by the addition of MNPs and drugs.

Rapid release of ions increases the pH around the tissue, which may hurt cell growth. Second, the destruction mechanism of these bioceramics is not precise. However, we believe that silicate ceramics are a promising window for future clinical applications considerable. High concentrations of Ca and Si, as well as high pH levels, inhibit cell growth and differentiation [53-61].

The obtained results of this study show that porous scaffolds made by the freeze-drying method in the vicinity of an EMF are able to absorb osteoblast cells with better bone growth ability at the implant site, which can improve the healing process more quickly. In the study of the amount of gentamicin release in the solution, it was determined that the concentration of calcium and silicon ions decreased with time on the scaffold surface in the early stages of immersion and has reached an almost constant value. These changes indicate the deposition of calcium compounds over time. As time passed due to the simultaneous dissolution of calcium ions, the concentration of calcium ions reached equilibrium and relatively stable.

CONCLUSION

The effect of MNPs on porous scaffolds is approved. According to the results, the mechanical properties of the magnetically reinforced samples are better than those of the previous ones without MNPs. The biodegradable system is designed using a double-penetration mechanism inside the gelatin-like body. The product obtained from the deposition of calcium phosphate phases inside the gelatin is a polymer-based nanocomposite with calcium phosphate-reinforcing particles inside it. Due to the mechanism employed in this design, the in-situ calcium-silicate-reinforcing phase is in situ. It was formed, and due to its chemical bonding with the background, it was well mixed. The sediment particles were distributed entirely in the field, and their dimensions were less than one micron with a geometric shape. The use of the spacer method resulted in regular and interconnected pores, and the size of the pores was within the proper range for bone cell growth. The mechanical properties of the scaffolds were within the range of the spongy bone. As two radical molecules collide, a single bond is formed between them, and the repetition of this can be seen by the presence of a magnet. It can be said that the MNPs are trapped in the gelatin-bioceramic polymer network and plays a physical cross-linker. Subsequently, the microscope images

of well-porous scaffolds

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this work are included in this published article.

FUNDING

This study started after receiving its scientific ethical approval from Isfahan University of Medical Sciences with evaluation of experimental and comparison investigation of bone filler that registered inquiry and funding under the No. 397398 and IR.MUI.RESEARCH.REC.1397.158.

CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

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