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Synthesis and Characterization of Bioactive Glass Nano-powders for Bone Tissue Regeneration

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Abstract

The present research aimed to synthesize the bioactive glass nano powder (MBG) through sol-gel process using lactic acid as a catalyst. The surface properties of the bioglass powders were investigated by scanning electron microscopy (SEM). Energy dispersive spectroscopy (EDS) was also used to estimate the elemental composition. The Fourier transformed infrared spectroscopy (FTIR) and the X-ray diffraction (XRD) revealed a crystallization associated to the formation of crystalline phases on the synthesized powders. The in vitro bioactivity was then studied at the key times during the different hours of immersion into acellular Simulated Body Fluid (SBF). The results showed the effects of lactic acid on powder microstructure. Moreover, the formation of biocompatible hydroxyapatite (HA) was studied on the surface of the samples immersed in SBF. The microstructure of the obtained hydroxyapatite layer was characterized using different methods. Based on the results, the morphology and microstructure of bioactive glasses were controlled by lactic acid. Bioactivity tests under laboratory conditions showed that lactic acid-derived glasses are capable of forming more smooth spheres of hydroxylapatite grains in comparison to the samples synthesized in the absence of the lactic acid. Altogether, the bioactive powders could play an important role in bone tissue engineering application.

Keywords*:* Bioactive glasses; Sol-gel; lactic acid catalyst; Hydroxyapatite formation; Bone regeneration

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1- Introduction

The introduction of bioactive glasses (BG) fabricated by Hench et al. in 1969 had a significant effect on the field of tissue engineering [1]. Today, Researchers show the advantage of the materials such as bioactivity, biocompatibility and biodegradability [1,2]. In the field of bone tissue reconstruction, the design of biomaterial with multi-purpose properties, such as injection, drug delivery, and biological activities, is one of the most crucial objectives [3]. Due to the least invasive nature, injected delivery system has been studied extensively. In addition, injected biological materials can improve tissue deficiency and irregular forms via bone reconstruction [4]. In this regard, material extrusion and its flow properties at micron-scale depend on its form. As a result, sphere and regular morphology facilitate the method of reconstructing bone tissue, optimize the drug release, and minimize the inflammatory reaction. Bioactive glass, due to its capability to attach to soft and hard tissue with minimal occurrence of inflammation and toxicity for the host, has been extensively utilized for bone tissue reconstruction [5]. Glass derived from the sol-gel process can be recognized as the third-generation pioneer biomaterial due to its capability to reconstruct and regenerate bone [6]. In addition, the biological activity of bioactive glass within the laboratory conditions has been illustrated by considering the amount of hydroxyl apatite crystal formation, which is the non-mineral part of human bone, after submerging into the simulated body fluid [7]. The microstructure and morphology of bioactive glasses have a significant effect on surface activity and cellular response and its surface activity is the reason behind the increased rate of bone reconstruction. Lei et al. [8] found that the addition of polyethylene glycol (PEG) to the sol-gel method can cause the rapid formation of BG microspheres. The influence of acid catalysts on sol-gel derived BG have been studied before [9]. The main aim of the present study is to synthesize bioactive glass nano powders (MBG) using the sol-gel process via lactic acid as a catalyst which developed for the reconstruction of bone tissue due to its unique porous structure and improved biocompatibility.

2- Experimental

1.2 Materials

The following materials were utilized in the project: absolute ethyl alcohol 99.7% (EA), hydrochloric acid (HA), lactic acid (LA), tetraethyl orthosilicate (TEOS, MERCK), triethyl phosphate (TEP, MERCK), calcium nitrate tetrahydrate (CN, MERCK), polyethylene glycol (PEG), and deionized water (DW). MBG (based on molecular composition, SiO_2 60%, CaO 36%, and P_2O_5 4%) was produced using the sol-gel synchronization method by using lactic acid as a catalyst.

2.2 preparation of MBGs

The flowchart of the sol–gel bioactive glass fabrication process is demonstrated in Figure 1. First, solution A was added to solution B slowly and then, mixed together for 2 hours. Next, polyethylene glycol was added in the process of mixing, and mixing was continued for another 1 hour. The resulted sols (solution C) were kept inside a 100mL Teflon glass at 60°C for about

1 day in the oven. After turning into the gel, the obtained gel was dried at 60°C for the duration of 24 hours and then, transferred into the furnace for about 2 hours at 600°C. The resulted MBG powders were tested without screening. The value of 0.05 for molar fraction of lactic acid to TEOS was obtained.

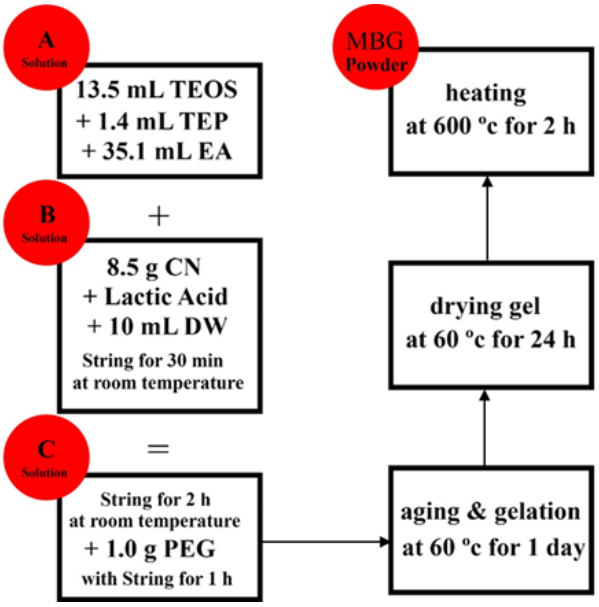

Figure 1: Flowchart of the sol–gel process.

3.2 Microstructure and in vitro bioactivity analysis

The surface morphology and microstructure of the synthesized bioactive glasses were investigated using a scanning electron microscope (SEM) (VP 1450, Germany). Before being observed, the particles were coated with gold for 5 min. The particle size distribution and chemical composition was analyzed by Energy dispersive X-ray spectra (EDS).

The in vitro bioactivity of the samples was tested in a simulated body fluid (SBF) described by Kokubo et al. [26-10]. To evaluate the biocompatibility of synthesized powders, simulated body fluid was utilized since ion density is close to human blood plasma. Table 1 shows the composition of SBF compared to human blood plasma. So, the particles were soaked in SBF at a concentration of 1 mg mL-1 in clean polyethylene bottles (200 mL volume), which had previously been sterilized using 70% ethanol and washed using deionized water [14]. The bottles were placed in an incubator at controlled temperature of 37 °C. All the samples were soaked in SBF for 24 h and 72 h without refreshing solution. Once removed from the incubation, the solids were separated by centrifugation, then washed three times with pure ethanol and deionized water, and finally dried at ambient temperature.

The formation of apatite layer on the surface of samples was observed by powder X-ray diffraction (XRD) (GNR-EXPLORER, Italy). The XRD analysis were performed with a Panalytical X'pert PRO diffractometer equipped Cu-Kα radiation (γ=0.154) in the 2θ range of 10^{o} to 70^{o}. Besides, the significant difference of the formation of bioactive apatite and the deposition rate on the resulted powders were investigated using Fourier transform infrared spectroscopy analysis (FT-IR) (AVATAR370).

3- Results and Discussion

By lactic acid-catalyzed sol–gel process, the bioactive glass microspheres (BGMs) were successfully prepared. Figure 2 shows the surface morphology and microstructure for MBGs derived through lactic acid-catalyzed sol-gel route. The lactic acid-derived glasses showed a regular sphere with micro with shallow micro-scratches. Analyzing the particle size of the samples illustrated that using lactic acid as a catalyst led to a narrow distribution domain and the hydrolysis of the silicon alkoxide and phosphorous alkoxide to achieve a homogeneous precursor solution [11,12]. The acid catalysts could cause the change in the morphology of the particles. Studies have reported [13,14,15] that acid catalysts with hydroxyl-carboxyl groups (such as acetic acid, citric acid and lactic acid) have an important effect on constructing nanostructures that related to the interactions of hydrogen bonds with the inorganic materials.

Except for the acid type, acid density is another influential factor in morphology and biocompatible glass microstructure. The suitable density of lactic acid can provoke and stimulate the formation of regular microspheres. Surface morphology and glass microstructure, submerged into the SBF, illustrated the formation of HA during 24h and 72h. After submerging during the determined time, the surface morphology of glasses changed and a layer of deposit was generated on the surface. Indeed, a continuous layer of compact particles merged on the surface of the powders. In addition, the size of deposits in all samples was observed to be between 50-150 nm.

Chen et. Al [16] fabricated the 58S bioactive glass by sol-gel method without using catalysts during the process. It has been shown that the morphology and microstructure of bioactive glasses were more controllable in the present research as compared with the current studies [16]. It is worth noting that the mentioned occurrence is related to the presence of lactic acid as a catalyst. The lactic acid-derived powders have had more regular spheres verified by SEM images. In addition, the EDS spectrum test illustrated the presence of suability components of synthesized bioactive glasses.

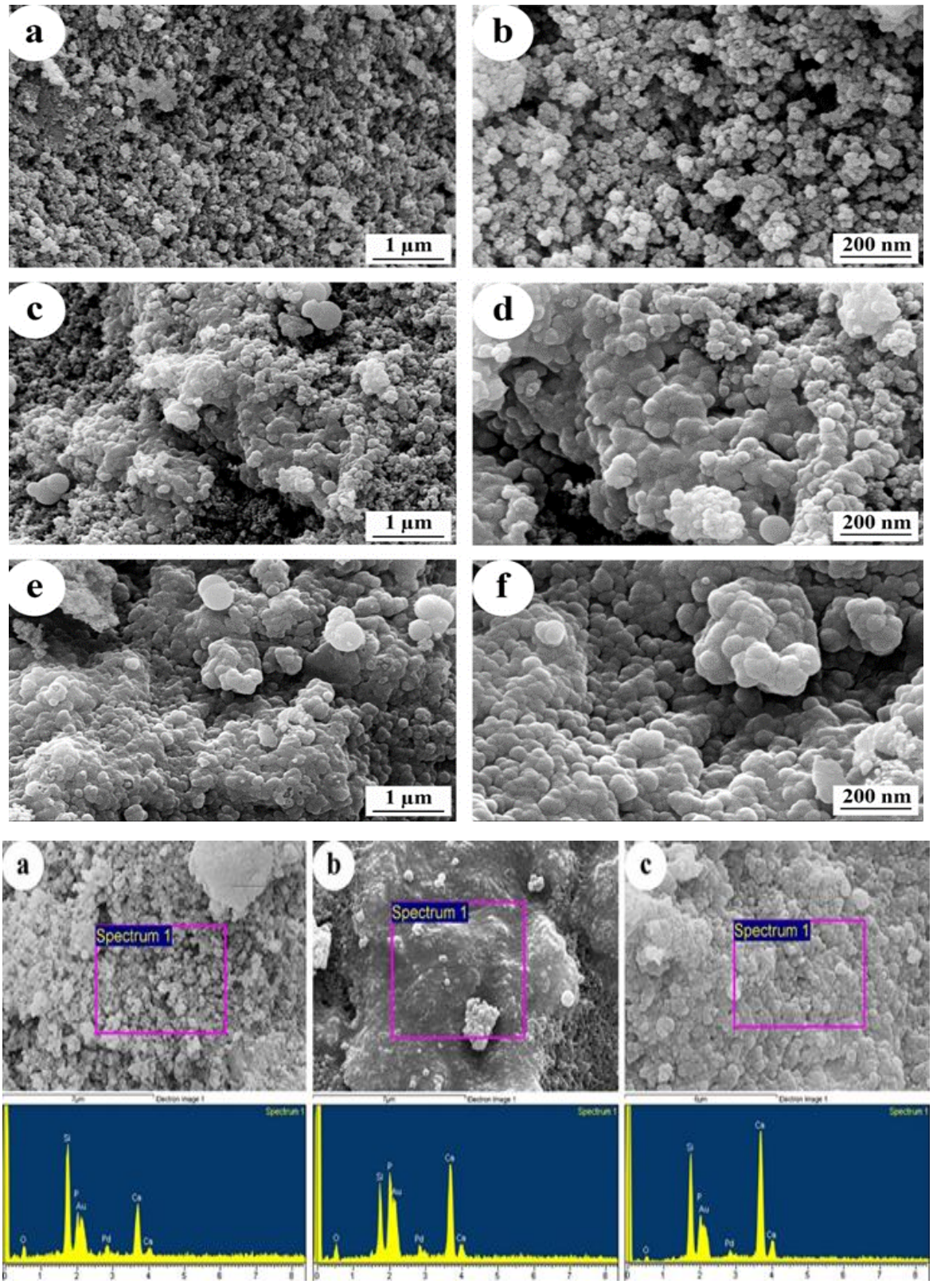

Figure 2: SEM and EDS of Bioactive glass After submerging into the SBF.

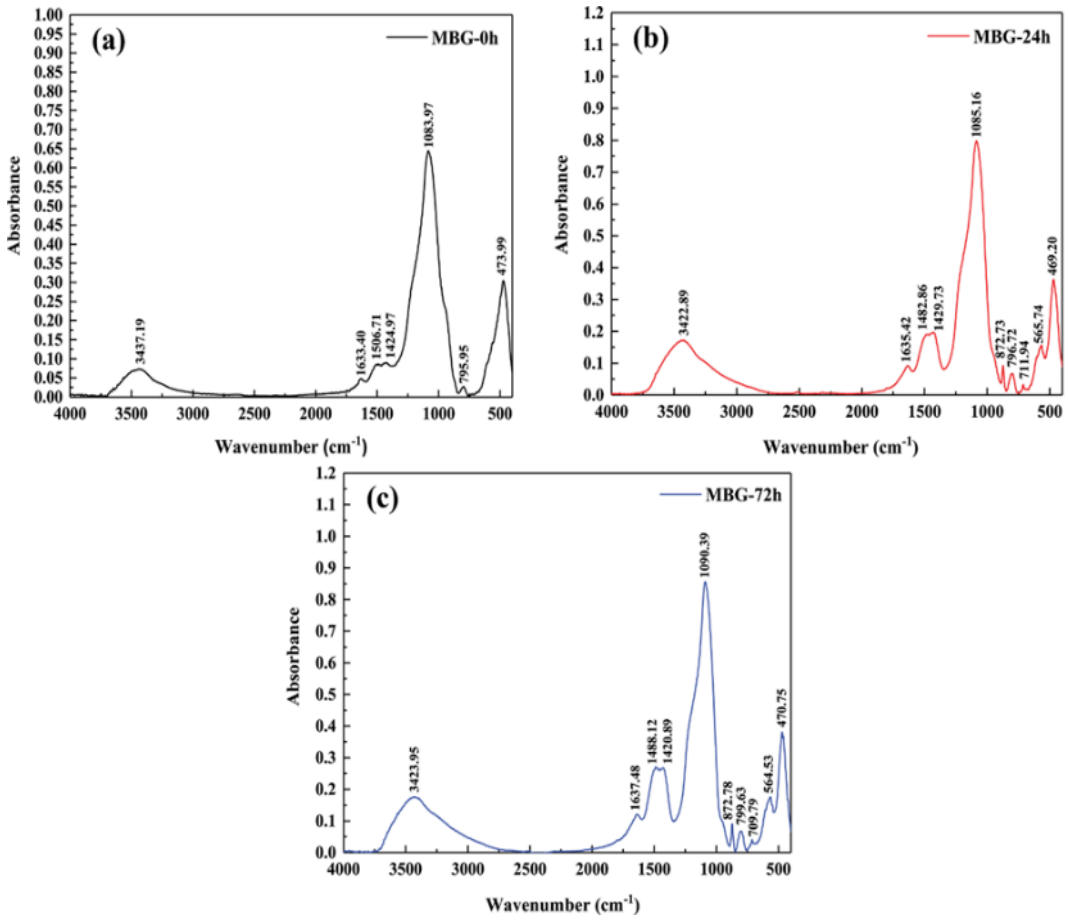

Figure 3: The FTIR spectra of the synthesized sol–gel glasses are exhibited.

To determine the significant difference in the formation of bioactive apatite, the amount deposit rate on the resulted powders was tested using the infrared spectrometer. FT-IR spectrum abstracted the samples before submerging and after submerging into the SBF during the duration of 24h and 72h. Figure 3 (a) shows the samples before submerging in the SBF media. As shown in Fig.3 (a), the spectra were similar and absorption characteristics of bands Si-O-Si illustrated special absorption at 1084 (stretching vibration), 796 (bending vibration), and 474 (bending vibration) cm^{-1}. Figure 3 (b,c) show the FTIR graphs of the samples after submerging into the SBF for the duration of 24h and 72h. The weak bending vibration bonds P-O were observed close to 565 and 711 cm^{-1}, and also, a peak was observed in 970 cm^{-1} which is related to bending vibration P-O. Also, absorption bands of carbonate groups were observed at 872 and 1442 cm^{-1}, which confirmed the stretching vibration of C-O, and illustrates the presence of apatite crystal layers. Also, the bond between 3300 and 3600 cm^{-1} was attributed to hydroxyls (O-H) vibration in hydroxyapatite (HA).

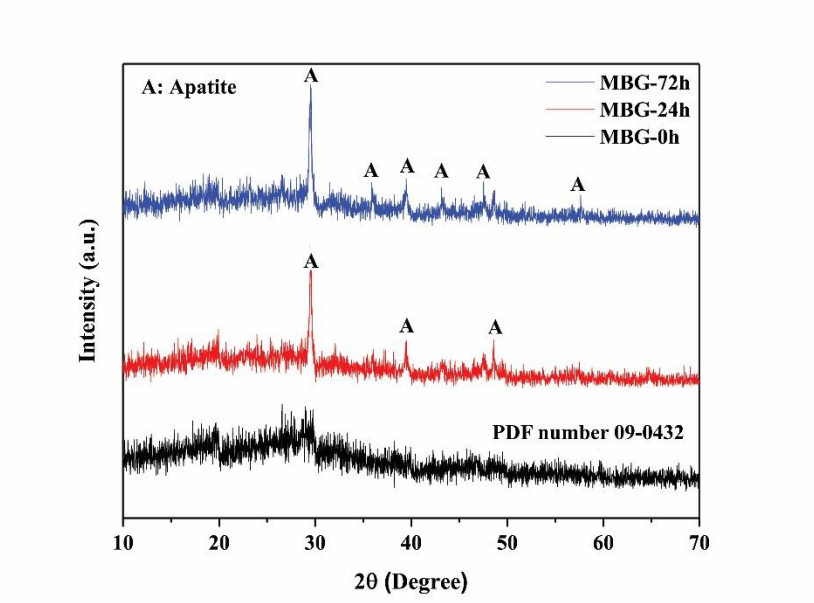

Figure 4 : XRD patterns of the Formation of apatite layer after submerging into the SBF.

Figure 4. demonstrates XRD patterns of the formation of the apatite layer after submerging into the SBF. Before submerging, only a small peak was identified between 20 to 30 degrees and this wide peak showed that bioactive glass was naturally amorphous. It also confirms that the sol-gel method is capable of generating bioactive glasses with an amorphous structure that doesn't contain any impurity [17]. After submerging into the SBF, all the samples during 24h and 72h illustrated the formation of an apatite phase on the surface. New peaks in 2Θ equal to 26, 32, 39, 46, 49, and 53 were attributed to planes (002), (211), (310), (222), (213), and (0014), which is the reflection of hydroxyapatite. The presence of identified peaks confirmed the formation of crystalline hydroxyapatite layer for all the samples. Cañaveral et. Al [18] fabricated a 58S bioglass modified with Mn element by Sol-gel method. They found that Mn contributed to the crystallization of the glasses at 700 °C. Montinaro et. Al [19] investigated the behavior of the three series of dense materials with different crystallization degree and phases fabricated by spark plasma sintering (SPS) from CaO-rich bioglass powders is investigated by soaking them up to 14 days in Simulated Body Fluid (SBF). They found that the completely amorphous materials produced after 2 min at 730 °C demonstrated the more pronounced substrate solution interaction. And they asserted that amorphous phase can cause the rapid generation (< 3 days) of an apatite layer on the substrate surface. According to Montinaro et. Al [19] the amorphous materials shows express substrate solution interaction more than crystalline and also our study proves it.

4- Conclusion

In this work, bioactive glass nanopowders (MBG) were synthesized using the lactic acid-catalyzed sol-gel method. The influence of the lactic acid as a catalyst on the surface microstructure and the formation of glass apatite phase were deeply investigated. According to the obtained valubale results, the presence of the lactic acid as a catalyst during the sol-gel process created microspheres with regular and relatively smooth form without any change in size. Also, the bioactivity test showed that all samples resulted in the formation of apatite layer during 24h and 72hours. Altogether, the synthesized bioactive powders could have a promising role in bone tissue engineering application.

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