Original Article

Bioactive Glass Nanopowder for theTreatment of Oral Bone Defects

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Abstract:

Objective: Osseous defects around dental implants are often seen when implants are placed in areas with inadequate alveolar bone, or around failing implants. Bone regeneration in these areas using bone grafts or its substitutes may improve dental implants prognosis. The aim of this study was to prepare and characterize the bioactive glass nanopowder and development of its coating for treatment of oral bone defects.

Materials and Methods: Bioactive bioglass coating was made on stainless steel plates by sol-gel technique. The powder shape and size was evaluated by transmission electron micropscopy, and thermal properties studied using differential thermal analysis (DTA). Structural characterization techniques (XRD) were used to analyze and study the structure and phase present in the prepared bioactive glass nanopowder. This nanopowder was immersed in the simulated body fluid (SBF) solution. Fourier transform infrared spectroscopy (FTIR) was utilized to recognize and confirm the formation of apatite layer on prepared bioactive glass nanopowder.

Results: The bioglass powder size was less than 100 nanometers which was necessary for better bioactivity, and preparing a homogeneous coating. The formation of apatite layer confirmed the bioactivity of the bioglass nanopowder. Crack-free and homogeneous bioglass coatings were achieved with no observable defects.

Conclusion: It was concluded that the prepared bioactive glass nanopowder could be more effective as a bone replacement material than conventional bioactive glass to promote bone formation in osseous defects. The prepared bioactive glass nanopowder could be more useful for treatment of oral bone defects compare to conventional hydroxyapatite or bioactive glass.

Key Words: Bioglass; Bioactive glass nanopowder; Bioactive glass coating; Bone defect

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INTRODUCTION

Bioactive glass is a non-resorbable biomaterial that its medical use evolved three decades ago, for its reported advantages of forming a strong bond with living tissues, including bone and soft connective tissue. This bonding is theorized to prevent fibrous encapsulation from occurring at the material interface. Bioactive glass is now clinically approved for use in dense form in non-load-bearing applications such as middle ear prostheses and endosseous ridge implants and as a particulate for periodontal defect repair [1,2]. Bioactive glass has potential as a bone replacement graft material and has effectiveness as an adjunct to intrabony defects surgical treatments [1-3].

Bone defects around dental implants are often seen when implants are placed in areas with

inadequate alveolar bone, (dehiscence defects, fenestration defects, residual intraosseous defects) in extraction sockets, or around failing implants. Bone regeneration in these defects by means of bone grafts or substitutes may improve the long-term prognosis of dental implants [4,5].

Guided tissue regeneration and bone fillers of various sizes and origins have been used to promote bone formation in osseous deformation, either before or in conjunction with endosseous dental implant placement [6]. Bioactive glass is a bioceramic that can be used as a particulate material. Animal experiments revealed a superior response to bioactive glass particles with small grain size range (300 to 355 µm) compared to hydroxyapatite granules. Osteoconductive bone formation starting from the wall of the defects was observed around the bioactive glass particles more than around hydroxyapatite particles [7].

Many clinical situations require implant placement in combination with bone grafts and/or other bone substitutes. The biomaterials employed include autografts, xenografts, allograft, and alloplasts such as bioactive glass. Several experimental models have demonstrated the ability of bioactive glass particles to promote osseous healing [8].

Single-tooth replacement with dental implants is a frequently used treatment option for the anterior maxilla. To ensure long-term success for osseointegrated implants, a sufficient bone quantity and quality should be present at potential implant sites. Hard tissue augmentation prior to implant placement may be a critical part of the treatment. Preservation of the alveolar process after tooth extraction is desirable because it facilitates placement of endosseous implants and minimizes adverse esthetic results associated with fixed partial dentures and implant-supported prostheses. Several local and systemic factors influencing the restorations of the bone volume following tooth extraction have been studied in experimental models. To preserve alveolar bone and abstain from residual ridge augmentation prior to implant placement, several particulate grafting biomaterials, including bioactive glasses, have been used immediately following tooth extraction to fill the socket [9].

After implantation of bioactive glasses particles, they undergo chemical transformation. This process leads to the formation of silica gel on the surface of the particles followed by the precipitation of amorphous calcium phosphate which will crystallize as hydroxicarbonate apatite by incorporating carbonates from the surrounding medium. A recent study has shown that the release of ions (Na, Ca, and Si) from bioglass material control the cell cycle leading to the differentiation and proliferation of bone cells, modulation of the expression of genes that regulate osteogenesis, and the synthesis of growth factors. Many researchers have demonstrated the value of bioactive glass particles as a filling material around dental implants, and their effectiveness in preserving alveolar ridge dimensions after the extraction of tooth [9].

Bioactive glass or bioglass can be used in granular form to fill bone defects. It has the capacity to bond to the osseous tissue; moreover, bone tissue repair and growth can be enhanced by its osteoconductive properties [10].

Bioactive bioceramics such as bioglass have gained access to great application successes in bone repairing [11]. Bioactive glass could elicit a specific biological in-vivo response at the interface and attach to the tissues, such as soft tissue and bone, with a strong chemical bond. This is the reason that bioactive glasses have been used for many different applications [12].

Certain compositions of bioactive glasses containing $CaO–SiO_2–P_2O_5$ bond to both soft and hard tissue without an intervening fibrous layer [13]. Bioactive glasses containing $CaO–SiO_2–P_2O_5$ have no local or systemic toxicity effect. No inflammation or foreign-body re-

sponse could be observed as a result of using bioactive glasses [13]. It has been recently shown that the cellular response of osteoblasts to bioactive glasses is a result of genetic control [14].

The recent trend in bioceramic research is focused on overcoming the limitations of bioceramics and in improving their biological properties via exploring the unique advantages of nanotechnology. The resorption process of synthetic bioceramic such as hydroxyapatite (conventional forms) is quite different from that of bone mineral. Bone mineral crystals are in nano-size with a very large surface area. These crystals grow in an organic matrix and have very loose crystal-to-crystal bonds; therefore, the resorption process by osteoclasts is quite homogeneous. Hydroxyapatite (micron size), on the contrary, present a low surface area and have strong crystal-to-crystal bond. In addition, the synthetic hydroxyapatite presents less bioactivity compared to bone mineral [15].

The grain size, large surface area to volume ratio, and the ultra fine structure of nanoscale engineering bioceramics similar to the biological apatite, provide surprising functional properties for these materials. This matter would have a great influence on the interaction between cells and implant in the body environment [16].

Greater surface area of the nano-bioceramic powders provides better sinterability and increased densification to decrease sintering temperature [17,18]. It seems that nanostructure bioceramic have better bioactivity compare to coarser crystals [19,20]. Nanostructure ceramics present an incomparable and promising character for orthopedic and dental implant formulations with better osseointegrative properties [21,22]. By controlling the structural and particle size in the range of nanoscale, some properties of bioactive bioceramic such as osseoconductivity, sintering characters, solubility and, mechanical reliability can be improved [23,24].

Keeping the aforementioned points in view, present study was aimed to produce and characterize bioactive glass nanopowder to provide a large bioactive surface for contact to living tissue and osseointegration, as well as for the treatment of oral bone defects.

MATERIALS AND METHODS

Sol-Gel preparation of bioactive glass:

The composition of the studied bioactive glass was; 57.44% CaO, 35.42% SiO_2 and 7.15% P_2O_5 in molar percentages and depends to the system of $CaO–SiO_2–P_2O_5$ [25,26].

The sol–gel precursors used in this study were Tetraethylorthosilicate (TEOS, Merck) which was selected as silica precursor for the sol, Hydrogen Ammonium Phosphate (Merck), and Calcium Nitrate (Merck). The silica sol prepared in alcoholic media with no catalyst. The initial procedure involved mixing TEOS and Ethanol as an alcoholic media. Distilled water was added to solution and allowed to mix until the solution became clear. The H_2O: (TEOS) molar ratio was 12:1. After 30 minutes, Hydrogen Ammonium Phosphate added to the stirring solution, and after another 20 minutes, Calcium Nitrate was added. The solution was then stirred for an additional hour.

On the completion of the hydrolysis procedure, the sols were aged in a drying oven at 50 ^oC to reach high viscosity near the gel point.

Bioactive glass Characterization:

Transmission electron microscopy (TEM) **(**CM200-FEG-Philips**)** was used to study the size and shape of bioactive glass nanopowder.

To study the thermal properties of prepared bioactive glass nanopowder, differential thermal analysis (DTA) in stagnant air atmosphere were carried out on the glass powder sample up to 1000 ^oC at an increasing rate of 5 ^oC /min.

X-Ray diffraction (XRD) technique (Philips X'Pert-MPD system with a Cu Kα wavelength of 1.5418 Å) was utilized to analyze

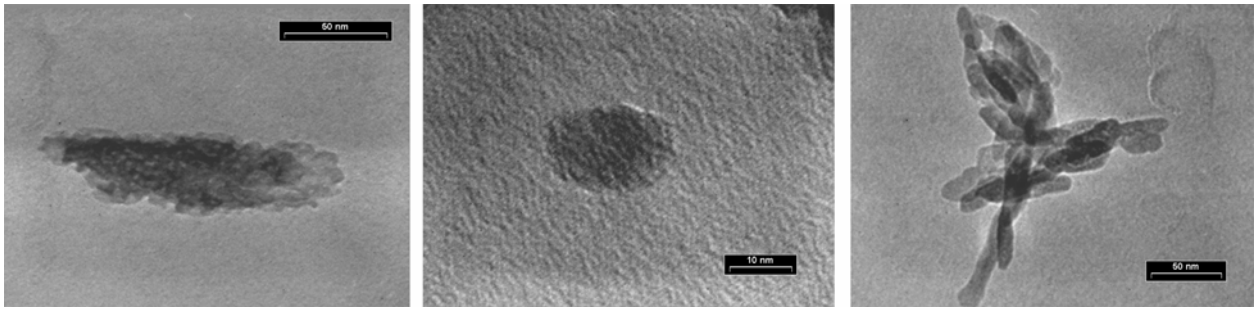

Fig 1. TEM images of the prepared bioactive glass nanopowder.

and study the structure and phase present in the prepared bioactive glass nanopowder. The diffractometer was operated at 40 kV and 30 mA at a 2θ range of º20–º70 employing a step size of 0.02.

***In vitro* bioactivity testing:**

Stainless steel 316L plates (2×2×0.2cm) were used as substrates. Specimens were cleaned in an ultrasonic bath and were immersed in ethanol. Then, they were dipped in the sol and were withdrawn at a rate of 5 cm/min. After holding at room temperature for 30 minutes, specimens were placed in an oven to heat dry at 300 ºC. According to DTA curve of prepared bioglass, the selected heating rate between 160-180 ºC and 260-280 ºC was 0.1 ºC/min. Finally the dried specimens were heat treated at 700 ºC. Scanning Electron Microscopy (SEM) and energy dispersive X-ray analysis (EDX) techniques (Phillips XL 30) were used to study the microstructure and morphology of the bioactive glass coating. EDX analysis was utilized to estimate the composition of the bioactive glass coatings.

Simulated body fluid (SBF) soaking was used to evaluate the *in vitro* behaviour of bioactive glass coated specimens. The composition of this buffer, described by Kokubo et al [27], has similar composition to human blood plasma. SBF is able to produce the same type of hydroxyapatite layers *in vitro*, such as that would form on the bioactive glass surface in the human body [27]. Bioactive glass coated specimens were immersed in SBF and total immersed specimens were placed in a water bath at 37 ºC for 21 days.

The formation of the apatite layer was identified, analyzed, and confirmed using Fourier transform infrared spectroscopy (FTIR) (Bomem, MB-100) in the range of 4000–100 cm^{-1}.

RESULTS

Characterization of bioactive glass:

TEM images of produced bioactive glass nanopowder that could be used for study of the size and shape of specimens are shown in Fig 1. The bioactive glass powder size was less than 100 nanometers, which is necessary to obtain superior bioactivity than coarser crystals, and prepare a homogeneous coating.

Fig 2 shows the DTA graph of the bioactive glass powder at the heating rate of 5ºC/min. Two exothermic peaks could be observed on the DTA curve at the range of 160-180 ºC and 260-280 ºC. Therefore, the selected heating rate of the powder specimens between 160-180 ºC and 260-280 ºC was 0.1 ºC/min.

The XRD pattern of the prepared bioactive glass nanopowder is demonstrated in Fig 3. This pattern confirms the formation of the bioactive glass nanopowder with amorphous structure. The XRD patterns of the prepared bioactive glass as the row material and after heat treatment at 900 ºC, 1000 ºC and 1100 ºC are shown in Fig 4. The peaks of the patterns matched the pattern of Larnite Ca_2SiO_4 (JCPDS#33-0302). One should notice that after heat treatment at 1000 ºC and 1100 ºC, the crystallinity is increased.

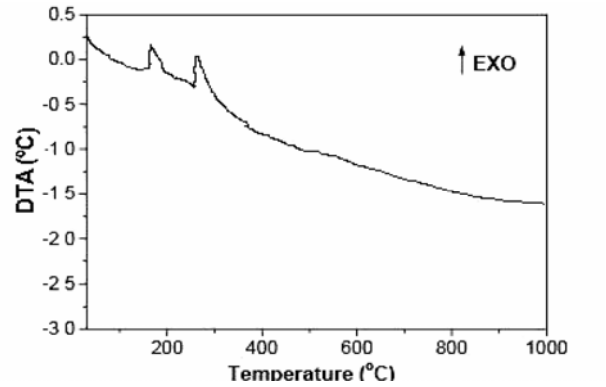

Fig 2. DTA curve for the bioactive glass nanopowder sample.

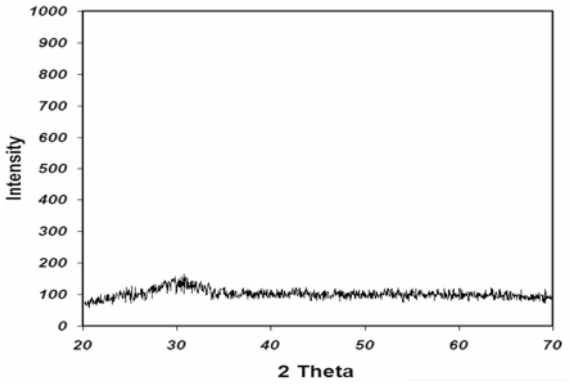

Fig 3. XRD pattern of the prepared bioactive glass nanopowder.

In vitro bioactivity evaluation:

The apatite layers on the bioactive glass coated specimens were further analyzed using FTIR, as shown in Fig 5. Well-defined phosphate peak (P–O stretching peak) could be observed at 1000-1200 cm^{-1}, indicating the formation of amorphous $CaO–P_2O_5$ rich layer. In addition, P–O bending peak at 550-620 cm^{-1} was also observed in the FTIR spectra. *In vitro* tests revealed that the bioactive glass coating induced the formation of a semi crystalline hydroxyapatite rich layer onto the bioactive glass coating surface as a result of the chemical reaction between this coating and surrounding body fluid, considered as preliminary signal of bioactivity after immersion in SBF for 21 days.

DISCUSSION

As it is showed in Fig 1, the bioactive glass powder size was less than 100 nanometers, which is necessary to achieve greater surface area, better bioactivity comparing the coarser crystals, and providing homogeneous coating. These results are in agreement with the result of previous studies [25,28].

For replacing or repairing osseous defects, using bioactive glass nanopowder seems necessary. All application of bulk bioactive glass in clinical use depends on its nano scale particles. Therefore, producing bioactive glass nanopowder is in the priority of other researchers' goals [28].

The sharp peaks of DTA graph of the bioactive glass powder show exothermic reactions, which occur between the range of 160-180 ºC and 260-280 ºC (Fig 2). These exothermic reactions could be attributed to releasing gaseous compounds from prepared bioactive glass during heating process. These reactions may cause cracks in the coating at these temperatures during drying of coated specimens. Therefore, the selected heating rates of the specimens at this temperature ranges, were very slow.

The XRD patterns indicated that the initial bioactive glass powder had amorphous structure (Fig 3), proving the sol-gel method could prepare pure glasses. As the heat treatment temperature of the prepared bioactive glass nanopowder increased up to 900ºC, only a diffraction broad peak existed in the pattern, showing that the bioactive glass nanopowder still had kept the amorphous state. By further increasing the heat treatment temperature, sharp peaks appeared at 1000 ºC, indicating that crystallization occurred in the bioactive glass (Fig 4). The Ca_2SiO_4 peaks became much sharper at 1100 ºC, attributing to further crystallization. The formation of the crystalline Larnite phase decreases the bioactivity of the bioactive glass [11], thus the coated specimens should be heat-treated at temperatures lower than the crystallization temperature.

In vitro tests and FTIR (Fig 5) investigation

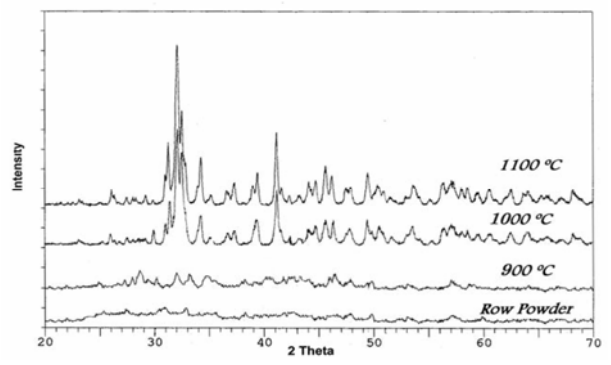

Fig 4. XRD patterns of the prepared bioactive glass sintered at various temperatures.

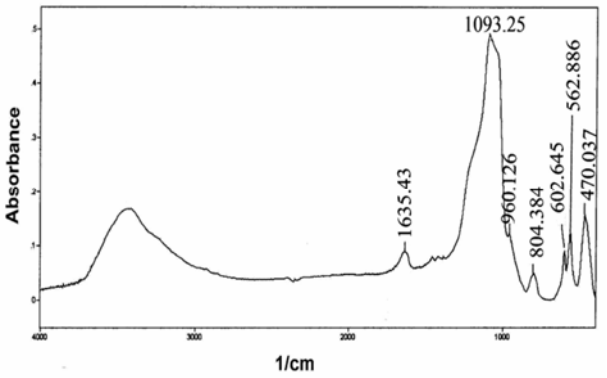

Fig 5. FTIR curve of the apatite layer formed on the bioactive glass coating.

revealed that the bioactive glass coating induced the formation of a semi crystalline hydroxyapatite (HA) rich layer onto the coating surface, which is a result of the chemical reaction between this coating and surrounding body fluid. This is considered as preliminary signal of bioactivity after immersion in SBF for 21 days; as a result, the prepared bioactive glass coating could be used as a bioactive surface layer on implants.

Bioactive glasses have demonstrated many advantages in different medical applications since they were introduced by Hench [28-30]. However, they can not be used for load-bearing applications because of their poor mechanical properties. One of the major applications of these bioactive glasses is for the coatings of metallic implants [31]. Bioactive glass coatings can obtain two purposes: enhancing the osseointegration of metallic implants, and protecting tissues from the corrosion products of the metallic implants [31].

Bioactive glass that consists of different glass compositions, and is able to bond to hard and soft tissue in a few hours, demonstrates a very good bioactive behavior [32]. Among the various coating materials, which are currently used in orthopaedic surgery, bioactive glasses provide better adaptation of the prostheses to bone cavity, prevent the fibrous tissue formation at the prostheses-bone interface, and grant a powerful chemical bond between implant and tissue [33].

The production of bioactive glass nanopowder is important for researchers from different aspects. The nano-sized structure of the powder not only has some advantages in coating the substrate through sol-gel method but also is important for its direct applications in the human body [26]. The degree of bioactivity in artificial materials similar to bone structure has constantly been taken into consideration by researchers. Different kinds of bioactive glass have been recorded with different percentages of components and the most prominent difference was related to the degree of their bioactivity. In order to use the bioactive glass as a replacing or repairing material for human bones, it should have a considerable degree of bioactivity [26,34,35].

In present study, the proposed composition for producing bioactive glass showed a noticeable degree of bioactivity. Complete crystallization of hydroxyapatite after 21 days of immersion of bioactive glass nanopowder in SBF solution indicated this fact.

Another advantage of bioactive glass nanopowder is the homogeneous and leveled coating of the solution containing these particles. In other words, the attainment of a uniform and homogeneous coating from the solution is indebted to the sol from which the glass particles are extracted [11].

Based on the results, the forthcoming study of

present authors is the in vivo evaluation of prepared bioactive glass nanopowder.

CONCLUSION

Bioactive glass nanopowder (57.44% CaO, 35.42% SiO_2 and 7.15% P_2O_5) was prepared by sol-gel technique. The bioactive glass powder size was less than 100 nanometers which is necessary to obtain greater surface area, better bioactivity, and preparing homogeneous coating. The formation of apatite layer after 21 days immersion in simulated body fluid confirmed the desired bioactivity of the bioglass nanopowder.

The prepared bioactive glass nanopowder could be more effective as a bone replacement material than conventional bioactive glass to promote bone formation in osseous defects and bone grafting to improve the long-term prognosis of dental implants.

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