Synthesis and Characterisation of Bioactive Glass 13-93 Scaffolds for Bone Tissue Regeneration

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Abstract: A modified sol-gel method was used in the current work to prepare a 13-93 bioactive glass powder, which was selected for the therapeutic actions of its constituent parts. In particular bioactive glass 13-93 can chemically bond with host tissue and induce osteogenesis. The produced gel was calcined at a temperature of 600 °C, while particle size analysis and x-ray diffraction were performed after the preparation of the glass powder. Porous bioactive glass 13-93 scaffolds were synthesised using the polymer foam replication technique that uses polyurethane sponges as a template. Sintering at 700 °C was then performed for one hour to produce the required structures. After sintering, the microstructure was examined by scanning electron microscope (SEM) and Fourier transform infrared analysis (FTIR). The x-ray diffraction (XRD) results were also examined. The average particle size of bioactive glass 13-93 thus produced was about 2.978 μm, and XRD pattern analysis showed that the porous scaffolds were amorphous. The microstructure of the 13–93 glass scaffolds contained interconnected cellular pores and a dense network of bioactive glass, allowing scaffolds with porosity between 80 and 83% to be obtained. An in vitro bioactivity test was performed on the scaffolds by soaking them in a solution of simulated body fluid (SBF). The subsequent SEM images confirmed the bioactivity of the prepared scaffolds based on the formation of obvious and dense hydroxyapatite particles on the surface after 7 days of immersion in SBF. It was thus concluded that bioactive glass scaffold prepared in this work via the polymer foam replication technique has the potential to be used in several future applications.

Keywords: 13-93 glass, scaffold, polymer foam replication, SBF.

1. Introduction

Bone abnormalities may occur due to the removal of tumours; birth defects, such as palate cleft; or trauma. Many bone graft procedures use auto grafting, extracting bone from elsewhere in the patient’s body, generally the pelvis; however, amount of bone available is thus limited, and donor site healing appears to be more painful and to take longer than treatment site healing [1].

Ceramics and glass are attractive bone repair scaffolding materials due to their ability to promote bone regeneration while bonding with surrounding tissue. During implantation, bio-glasses slowly convert to hydroxyapatite, a bone mineral component, creating an osteoinductive effect [2]. Bio-glasses are thus commonly selected surface reactive biomaterials for use in human bodies as implants for restoring and replacing damaged or diseased bones due to this bioactivity. A surface layer of hydroxyapatite has been observed to be formed when bioactive glasses are inserted in a human body or immersed in a solution of simulated body fluid (SBF), and as hydroxyapatite's composition is similar to that of bone, it enables better bone grafting [3].

Such glass can be synthesised using either sol-gel or melting processes. The first bio-glass (24.4% Na2O, 46.1% SiO2, 2.6% P2O5, and 26.9% CaO) was named bioactive glass, and this is now a common commodity, being used as bone filling material in particle form [4, 5]. This bioactive glass is mostly synthesised using traditional melting approaches that involve heating the raw materials in a precursor mixture and after then applying quenching of the molten product to produce a bio-glass. However, in
recent years, several efforts have been made to move the manufacture of bio-glass towards the sol gel method. The sol-gel approach provides various benefits over traditional melting techniques. It allows extremely fine powders to be produced, using a highly regulated composition that necessitates only moderate processing temperatures in order to produce bioactive glass with higher purity and homogeneity than that produced using the traditional melting technique; the sol-gel method also assists with the regulation rates of ion dissolution in physiological solutions [6].

Two specifications for the design and characterisation of the manufactured scaffold are required. The first is that the scaffold should exhibit sufficient mechanical characteristics to match those of the tissue at the implantation region, while the second is that it should enable the mass transportation of nutrients and oxygen to allow the new tissue to expand and vascularize [7]. Several techniques have thus been employed in the development of porous 3D bioactive glasses and polymer scaffolds, including solid freeform processing, thermal induction by phase separation, freeze casting, solvent leaching, and replication using polymer foam [2].

Among these methods, polymer sponge replication, which uses the polyurethane sponge as a prototype to create a customised scaffold with controllable pore size [8], has shown particular promise. Particular attention has been paid to impregnating the polymer foam with suspensions that include ceramic powder particles and suitable additives, as porous ceramics offer certain special properties, such as good permeability, high specific surface areas, very low thermal conductivity, and low density. Any scaffold must be biocompatible, offer high pore interconnection, and have controllable rates of degradation to encourage bone cell growth [9]. In general, the use of PU foams allows high porosity levels (∼90 %) to be obtained, though the mechanical properties of glass scaffolds are usually in the lower reference range considered for trabecular bone [10]. The suitability of a scaffolds for bone tissue engineering applications varies dependent on their structural parameters such as total porosity, pore size, and pore interconnectivity, and thus these features must be considered when producing a BTE scaffold, which should ideally exhibit a total porosity of between 50 and 90% [11].

In this study, the production of a bioactive glass 13–93 dependent porous scaffold was done by means of a polymer sponge replication technique, based on the restrictions related to many other techniques. These scaffolds, which were designed with a microstructure similar to that of human trabecular bone, were then used in order to study bone regeneration support from this glass type.

2. Experimental procedure

2.1. Synthesis of bioactive glass 13-93 powder

The composition of the 13–93 bio-glass used in this work was 53SiO2, 12K2O, 20CaO, 6Na2O, 4P2O5, and 5MgO by weight percent. The sol-gel technique was used to prepare the 13-93 bio-glass from the raw materials as shown in Table 1.

At room temperature, 40 ml of TEOS was added to 0.3 M of HNO3 aqueous solution. The H2O/TEOS molar ratio was maintained at 15. To encourage hydrolysis, the mixture was stirred for about 1 hour. After that, each chemical compound (1.9 ml of TEP, 3.34 g of NaNO3, 6.32 g of Mg(NO3)2, 6H2O, 16.54 g of Ca(NO3)2·4H2O, and 5.23 g of KNO3) was added in sequence as the mixture clarified from the addition of the previous compound; the resulting mix was then stirred for another hour at the same temperature [12]. The resultant transparent mixture was continuously stirred at room temperature in a closed flask overnight, to permit homogenisation, and the solution was kept at the same temperature until gelation occurred after two days. Tetraethyl orthosilicate undergoes reaction hydrolysis during sol formation, thus forming groups of silanol. The gel was then aged at 70 °C for two days, then dried for 24 hours at 120 °C. The aqueous gel began to create a silicate structure during this ageing process, allowing formation of a glass structure. The produced gel was crushed in a ball milling device, then subjected to calcination treatment at a temperature of 600 °C for 3 hrs in order to extract the structural nitrates.
Table 1: Materials used for the preparation of 13-93 glass

| No. | Material                  | Chemical formula | Molecular weight (g/mol) | Purity (%) | Manufacturer        |
|-----|---------------------------|------------------|--------------------------|------------|---------------------|
| 1   | Tetraethyl orthosilicate  | Si(OCH₃)₄        | 208.3                    | 99         | Sigma-Aldrich, Germany |
| 2   | Triethyl phosphate (TEP)  | (CH₃CH₂O)₄PO     | 182.2                    | 99         | Sigma-Aldrich, Germany |
| 3   | Potassium nitrate         | KNO₃             | 101.1                    | 99         | Thomas Baker, India |
| 4   | Sodium nitrate            | NaNO₃            | 84.99                    | 99         | Thomas Baker, India |
| 5   | Calcium nitrate tetrahydrate | Ca(NO₃)₂.4H₂O   | 236.2                    | ≥98        | Himedia, India      |
| 6   | Magnesium nitrate         | (Mg(NO₃)₂.6H₂O)  | 256.4                    | ≥98        | Himedia, India      |
| 7   | Nitric acid               | HNO₃             | 63.01                    | 69-72      | CDH, India          |

2.2 Scaffold fabrication
The scaffold for the 13-93 bioactive glass was synthesised using polymer sponge replication. Polyurethane sponge samples were obtained from industrial sources, and glass powder and binder poly ethylene glycol (PEG) were used to prepare various weight percentages of glass slurry. Aqueous suspensions containing 70 wt.% glass particles, and 2 wt.% PEG were determined to be optimal, as the resulting scaffolds had sufficient strength to resist manual handling. Cubical (1×1×1 cm³) sponge specimens were immersed in the slurry for 12 hours to allow adequate absorption of glass particles, squeezing of the polyurethane sponge was then done to expel any excess slurry.

The soaked sponge samples were then oven-dried for 10 h at 80 °C and sintered for one hour at 700 °C at a 3 °C/min heating rate. After that, the scaffolds were cooled to room temperature slowly by switching off the furnace and leaving them in place. The sintered scaffolds were then used for examination of scaffold microstructure, and in vitro evaluations.

2.3 Characterisation
The particle sizes of the synthesized 13-93 bioactive glass powders were obtained via a particle size analysis device (90 Plus laser particle size analyser) at the University of Technology, Nanotechnology and Advanced Materials Research Centre. Both the starting powders of the glass and the sintered scaffolds were analysed using x-ray diffraction (XRD-6000, NF type) operated at 35 KV, 40 mA, using Cu-Kα radiation with a step size of 0.05° over an angle of 20 and a range of 10 to 90° to distinguish the various phases present. The various interaction and chemical bonds were identified using FTIR microscopy [BRUKER, Germ] within the range of 600 to 4,000 cm⁻¹. Microstructural analysis of the scaffolds was performed using scanning electron microscopy (SEM) (VEGA3 TESCAN Performance in Nanospace). Before SEM examination, the scaffolds were coated with a thin layer of Au-Pd to achieve adequate surface conductivity and to prevent surface charging.

The porosity of the scaffolds was measured based on the obtained density by using equations (1) and (2) [13]:

\[
\text{porosity} \% = 1 - \left( \frac{\rho_{\text{relative}}}{\rho_{\text{Bulk}}} \right) 
\]  

(1)
\[ \rho_{\text{relative}} = \frac{w_d}{\text{volume of scaffold}} \]  

(2)

where \( \rho_{\text{relative}} \) and \( \rho_{\text{bulk}} \) are the relative and bulk densities (g/cm\(^3\)), respectively; \( w_d \) is the dried weight of the specimen; and volume of scaffold is in cm\(^3\).

Glass 13-93 scaffolds were soaked in SBF at 37 °C after analysis, as one indicator of bioactivity is the creation of a calcium phosphate thin film. The solution of simulated body fluid used was created as per Takadama and Kokubo [14]. The cubic glass scaffolds were washed with ethanol in the ultrasonic container, dried at room temperature, and placed in wrapped polyethylene flasks containing 50 ml of SBF at 37 °C. The solution of simulated body fluid was changed every two days, then after the selected duration of immersion, the scaffold samples were dried in air for 24 hours before examination by SEM.

3. Results and discussion

3.1 Characterisation of synthesis powder

Figure 1 (a) shows the gel of 13-93 glass after about 48 hours aging and drying. After calcination treatment at 600 °C for 3 h, the glass powder shown in (b) results.

![Figure 1](image.png)

**Figure 1.** Photographs of glass prepared by sol-gel (a) gel formation, (b) glass powder after calcination.

3.1.1 Particle size analysis

The particle size of the bioactive glass powder was measured after milling, with the average particle size identified as being about 2.978 \( \mu \text{m} \), as shown in figure 2.

Effective Diameter: 2.978 \( \mu \text{m} \)
Polydispersity: 0.313
Baseline index: 0.0 / 95.61%
Elapsed Time: 00: 01: 30
3.1.2 XRD analysis

X-ray diffraction of the glass 13-93 powders prepared by sol-gel after calcination is shown in figure 3. Based on an analysis of the patterns, diffraction crystallisation is not apparent, and the material does not display any phase crystallisation. The diffraction peaks are as seen in the normal amorphous phase of glass, with a wide band at 2θ between 25° to 35° [15].

3.2 Characterisation of scaffold fabrication

3.2.1 XRD analysis

The XRD patterns of glass 13-93 scaffolds show similar patterns, supporting the amorphous nature of the powders, even after sintering at a temperature of 700 ºC, as shown in Figure 4. The values are highest at about 2θ ~35°. It has been observed that crystallisation prior to implantation of 13-93 bioactive glasses might slow down any resulting mineralisation [15].
3.2.2 Microstructure properties

Figure 5 shows the SEM images, highlighting the interconnected porous nature of the glass scaffold after sintering. The morphological and microstructural features of glass scaffolds were found to be identical to the human trabecular bone, as shown in figure 6. The microstructures of the polyurethane sponge foam used in these experiments were also similar to those of human trabecular bone.

Figure 5. SEM showing the porous nature of glass 13-93 scaffolds after the sintering process.
3.2.3 Porosity Determination

A porous structure is required for use in scaffolds to improve mechanical interlocking between the implanted biomaterial and the surrounding natural bone [16]. The porosities of the 13-93 bioactive glass scaffolds were thus calculated using equation (1), with the results showing about 80 to 83% porosity. This is in agreement with the morphological observations as illustrated in Figure 5.

3.2.4 FTIR analysis

The FTIR spectra of the bioactive glass 13-93 scaffold after sintering are shown in figure 7. Although though the complex peaks in the range 1,200 to 900 cm\(^{-1}\) may be the result of combined PO and SiO groups, the bands at 1,040 and 1,097 cm\(^{-1}\) were an extension vibration formula of Si bonded to non-bridging oxygen. The vibration bending of Si-O-Si bonds was also observed in the spectra based on the measurement at ~470 cm\(^{-1}\). In addition, assuming the presence of Na\(^+\) or other cations, the slight peak at ~760 cm\(^{-1}\) in the spectra may be caused by Si-O-Si, while resonances at ~600 cm\(^{-1}\), correlated by the P-O vibration bending of PO\(_4^3\), were clear.
3.2.5 Microstructure properties after immersion in SBF

Figure 8 shows the SEM micrographs of 13–93 bio-glass scaffolds after 7 days soaking in a solution of simulated body fluid. This treatment in the SBF at 37°C produced a fine particulate surface layer, created by scaffolds’ contact with the SBF solution, which meant that the surfaces of the scaffolds were fully covered with globular aggregates with a common morphology with the apatite.

Figure 8. SEM images of scaffolds showing spherical aggregates of apatite after immersion in SBF
4. Conclusions
Porous bioactive scaffolds were successfully prepared via the foam replication technique, producing microstructures similar to those of trabecular bone. It was thus shown to be possible to produce porous scaffold with homogeneous pore distribution. The apparent porosity of the resulting 13-93 bioactive glass scaffolds was calculated at 80 to 83%, and robust bioactivity was observed such that the apatite layer needed only 7 days to complete formation on the scaffolds’ surfaces. These results support the promising potential of these scaffolds for use in the repair of bones.

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