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# Mechanical properties, in vitro and in vivo bioactivity assessment of $Na_2O$-CaO-$P_2O_5$-$B_2O_3$-$SiO_2$ glass-ceramics

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ABSTRACT

Glasses having chemical composition based on $Na_2O$-CaO-$P_2O_5$-$SiO_2$ system were crystallized. Then, the resultant crystallized phases were examined by X-ray diffraction technique. Furthermore, density, microhardness and fracture toughness were measured. In order to investigate the biological responses of these glass-ceramic samples, in vitro and in vivo experiments were performed. In vitro test was performed by soaking the prepared samples in simulated body fluid (SBF) for different time intervals and then, specimens were examined by Fourier transform infrared spectroscopy. Moreover, the conversion kinetics of these samples to hydroxyapatite (HA) were determined by measuring the weight loss of glass-ceramic grains, pH values of SBF solution and recording the ionic concentrations of Si, B, P and Ca using inductive coupled plasma-atomic emission spectroscopy. The results pointed out that the prepared samples possessed fair in vitro bioactivity. However, after six weeks of implantation, the prepared glass-ceramics, on the contrary to the parent glasses, did not exhibit any bioactivity suggesting that they may need longer time. On the other hand, the crystallization process caused significant increases of microhardness and density values. From these results, we can conclude that the prepared glasses and glass-ceramics had suitable properties for bone grafts and dental applications, respectively.

## 1. Introduction

Recently, there is a growing need for development of glass-ceramics to be used in biomedical applications due to their promising properties such as bioactivity and appropriate mechanical properties. Furthermore, they exhibit other vital properties in healing process as they are also anti-inflammatory, antimicrobial and angiogenic agents [1]. They can be easily fabricated by heat treatment of glasses with suitable compositions and consequently, they are subjected to controlled crystallization to the lower energy and crystalline state [2,3]. It is worth to mention that some important points should be put in our consideration to obtain typical bioglass-ceramic such as the choice of appropriate glass composition, where some types are too stable and accordingly; they cannot be crystallized, and heat treatment should be carefully adjusted to keep acceptable properties of the obtained glass-ceramics [4–6]. As a general consideration, glass-ceramics are not fully crystalline but their typical microstructure is mainly ranged from 50 to 95 vol% crystalline and the remainder being residual glass [7]. Notably, their bioactivity is significantly retarded, and in some cases, turn bioactive glasses into inert materials. This bioactive character is mainly depending upon the initial glass composition, sintering process and the resultant phase composition [8]. However, if the resultant glass-ceramic doesn't exhibit bioactivity, it can be used as a durable material in restorative dentistry [7].

It is well-known that the ability of bioceramics to form hydroxyapatite (HA) or carbonated hydroxyapatite (CHA)-like layer on their surfaces is considered as indication for their bioactivity. However, this ability is greatly dependent on the type of the used physiological buffer. It is important to note that the calcium and phosphate concentration levels in the buffer can negatively or positively affect the apatite formation. Since the composition of SBF solution is close to the saturation level of apatite, any exposed surface to this solution can easily form apatite layer irrespective of whether it is bioactive or not [9,10]. Accordingly; some materials such as dicalcium phosphate dehydrate can form HA layer in vitro but they cannot directly bond to living bone. In contrast, β-TCP doesn't frequently form such layer in SBF solution but it is able to form HA layer in vivo [9,11]. Therefore, in order to explore bioactivity of any material, in vivo experiments should be carried out.

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**Table 1**
Nominal composition (wt%) of the parent borophosphosilicate glasses.

| Glass code | Chemical composition (mol%) | | | | |
|---|---|---|---|---|---|
| | $Na_2O$ | CaO | $P_2O_5$ | $B_2O_3$ | $SiO_2$ |
| **G1** | 20 | 15 | 45 | 5 | 15 |
| **G2** | 20 | 15 | 40 | 10 | 15 |
| **G3** | 20 | 15 | 35 | 15 | 15 |
| **G4** | 20 | 15 | 30 | 20 | 15 |
| **G5** | 20 | 15 | 25 | 25 | 15 |

## 2. Experimental work

### 2.1. Glass preparation

Melt quenched $20Na_2O$-15CaO-$15SiO_2$-$xB_2O_3$-(50-x)$P_2O_5$ glasses with x = 5, 10, 15, 20 and 25 mol% have been prepared from starting materials of analytical reagent grade. Silicon oxide ($SiO_2$), sodium carbonate ($Na_2CO_3$), calcium carbonate ($CaCO_3$), boric acid ($B_2O_3$) and ammonium dihydrogen phosphate ($NH_4H_2PO_4$) powders were thoroughly mixed in the appropriate quantities and, then, ground in an agate mortar. The weighed well-mixed batches were melted in a porcelain crucible in an electric furnace at 1250–1300 °C for 2 h in air. The molten liquid was occasionally stirred to ensure homogeneous mixing of all constituents and to obtain bubble-free samples. The glass, formed by quenching the melt on a stainless-steel mold, was immediately transferred to another muffle furnace where it was annealed at about 380 °C for 1 h. Then, the muffle was switched off and the temperature decreased to room temperature with a rate of 25 °C/h. The nominal compositions of the prepared glasses together with their abbreviations; namely G1, G2, G3, G4 and G5 are given in Table 1.

### 2.2. Differential thermal analysis (DTA)

DTA measurement was carried out on powdered bioglass samples which were examined up to 1000 °C and the heating rate was 10 °C/min. The DTA data were used to obtain the proper heat treatment temperatures to obtain the corresponding glass–ceramic derivatives with high crystallinity. It is worth to note that the abbreviations of the obtained glass-ceramic samples are S1, S2, S3, S4 and S5 which correspond to G1, G2, G3, G4 and G5, respectively. Briefly, these results showed that the values obtained were for glass nucleation ($T_g$) and the crystallization ($T_c$) temperatures.

### 2.3. X-Ray diffraction (XRD)

The crystalline phases of the formed glass-ceramic samples were investigated by XRD technique (Philips PW "1373" X ray powder diffractometer with Cu K-Ni filtered radiation) and the obtained data was interpreted based on JCPDS X-ray diffraction card files.

### 2.4. Density and mechanical properties

Density was measured for each sample using Archimedes method with water as the immersion liquid. The following formula was used to calculate the density:

$$\rho = \frac{W_{air}}{W_{air} - W_{water}} \times \rho_{water} \quad (2.1)$$

where $W_{water}$ and $W_{air}$ are the weight of samples in water and air, respectively and $\rho_{water}$ is the density of water.

Vickers microhardness (*Hv*) of the 2 mm thick polished samples was measured with a Shimadzu-HMV (Japan) microhardness tester using 100 g load under ambient laboratory conditions with a constant indenter dwell time of 15 s. The hardness was calculated using Eq. (2.2), by finding the ratio of the applied load to the pyramidal contact area of the indentation [12,13].

$$H_v = 1.854\frac{P}{D^2} \quad (2.2)$$

The fracture toughness, $K_{IC}$, of the samples was determined from the indentation fracture using Vickers microhardness tester. $K_{IC}$ was calculated using the Eq. (2.3) [14,15]:

$$K_{IC} = 0.016H_V\frac{a^2}{c^{3/2}} \quad (2.3)$$

### 2.5. In vitro studies in simulated body fluid (SBF)

Generally, SBF is used to assess the in vitro bioactivity of the materials by soaking them in this solution for different time intervals. Kokubo et al. [16,17] described the preparation method of this solution. As discussed earlier in Ref. [18], the ratio of 0.01 g/ml is suggested to achieve excess SBF volume that surrounds the glass grains. Accordingly; glass-ceramics were ground in agate mortar and sieved to obtain fine particles in the size range of 106–180 μm and used for bioactivity measurements. The powder samples were placed in polyethylene bottles containing 50 ml of SBF at 37 ± 0.5 °C for 3, 7, 14 and 30 days. Then, the samples were taken out of the bottles and dried at room temperature. The formation of HA-like layer on the surfaces of soaked glass-ceramic powders was followed up by FTIR spectroscopy.

#### 2.5.1. FTIR studies

FTIR spectra of the glass-ceramics, before and after soaking, were obtained at room temperature using the KBr pellet method by an infrared spectrophotometer type (Vertex 70) in the wavenumber range of 2000–400 $cm^{-1}$ using 100 scans at 2 $cm^{-1}$ resolution.

#### 2.5.2. The pH measurements

In this work, pH meter (Jenway 3510) was used to record the pH changes of SBF solution that were occurred as a result of soaking of these specimens for different time periods. In order to ensure the reproducibility of the readings, three measurements were performed for each time.

#### 2.5.3. Weight loss measurements

The weight loss percentage after an immersion time (*t*) can be calculated from the following equation:

$$\text{Weight loss\%} = \frac{W_i - W_t}{W_i} \times 100\% \quad (2.4)$$

where $W_i$ is the initial weight of glass-ceramic powder (i.e. before soaking) and $W_t$ is the weight of dried glass-ceramic powder after soaking in the SBF for a time (*t*). The measurements of weight loss were carried out in triplicate.

#### 2.5.4. Inductive coupled plasma-atomic emission spectroscopy (ICP-AES)

Ca, P, B and Si ion concentrations in the SBF solution after soaking of the investigated specimens were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, model Jobian Yvon Horiba Ultima 2000).

### 2.6. In vivo studies

#### 2.6.1. Animals and grouping

Thirteen (15 male and 15 female) 8-weeks old albino rats weighing about 150–250 g were used in the present study at surgery, anaesthesiology and radiology department, Faculty of Veterinary medicine, Cairo University (Egypt). The experimental animals were distributed randomly into 2 groups.