Journal of Non-Crystalline Solids xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Non-Crystalline Solids

journal homepage: www.elsevier.com/locate/jnoncrysol

In vitro biocompatibility evaluation of canasite-calcium phosphate glass-ceramics

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ARTICLE INFO

Keywords:
Canasite
Calcium phosphate
Double-phase glass-ceramic
Bioactivity
Biocompatibility

ABSTRACT

This research introduces a new bioactive glass-ceramic material based on canasite and calcium phosphate with different molar ratios in the range from 2 to 0.5. The glass-ceramics were obtained by controlled two-stage heat-treatment of the parent glass (encoded G) at different temperatures (700, 800, 900 and 1000 °C, encoded C700, C800, C900 and C1000, respectively). The glass and glass-ceramics were characterized by DTA, XRD, FTIR and SEM. The four point bending strength was also measured. In vitro bioactivity of the selected samples was investigated in SBF, and the cytotoxicity test was performed by culturing the samples with normal human lung fibroblast cells (WI-38 cells) and baby hamster kidney cell line (BHK cells). The results of XRD analyses showed that crystalline phases of frankamenite/canasite-A and fluorapatite were detected, and xonotlite crystal phase was formed at high heat-treatment temperature. The bending strength was significantly affected by the heat-treatment temperature. It increased with increasing crystallization temperature. SEM micrographs showed a homogeneous microstructure of interlocked needle- and rod-like crystals. Moreover, immersion of the samples in SBF demonstrated that the samples G and C900 showed a relatively better ability than other two samples. The results of the cytotoxicity test showed that cytocompatibility behavior of the samples was varied with the type of cell line. G sample showed a significant higher viability than C900 sample with the WI-38 cell line for all sample concentrations, while, this significant viability differences were not observed at low and high sample concentrations cultured with BHK cells.

1. Introduction

The demand of synthetic materials for fixation, reconstructing and repairing broken and damaged bone tissues is progressively increasing. These materials are known as synthetic bioactive materials such as calcium phosphate and magnesium phosphate ceramics, bioactive glasses and bioactive glass-ceramics, which have been used widely in different biomedical applications. Bone substitute materials for repairing bone are necessary if self-healing is not effective during the recovery. Preparation of ideal bone substitutes should have good biocompatibility and bioactivity; adjustable degradation and absorption rates. In an ideal case all physical and biological properties should be similar to the natural bone. In principle, this can be achieved by a similar chemical composition, structure and shape. Nowadays this aim is not yet reached, but the surface morphology should be suitable for cells' adhesion, proliferation and differentiation and the mechanical properties should be equivalent to or better than those of natural bone [1]. Bioactive glasses and glass-ceramics have found potential applications in various medical fields such as orthopedics, ossicular implantation for alleviating conductive hearing loss dentistry and maxillofacial applications [2,3]. Nevertheless, most bioactive glasses have a comparatively low flexural strength, low fracture toughness and they are brittle due to the amorphous nature of glass [4]. This means that the mechanical strength of these bioactive glasses is inadequate to be used in load bearing applications, and hence it restricts their further clinical utilization. Therefore, the improvement of the mechanical properties of bioactive glass is required. One promising way is to transform the glass into glass-ceramics. Glass-ceramics are polycrystalline solids prepared by controlled crystallization of glass via a heat-treatment process. They contain one or more crystalline phases and in most cases, a residual glassy phase [5]. Glass-ceramic has received great attention in the biomedical applications because it combines the bioactive features of bioactive glass and the advantageous mechanical properties of ceramic materials. Hence, glass-ceramic should be more suitable for bone replacement than the conventional bioactive glass materials.

Bioactive silicate glass-ceramics are mostly used due to their

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https://doi.org/10.1016/j.jnoncrysol.2018.03.003
Received 8 January 2018; Received in revised form 17 February 2018; Accepted 3 March 2018

comparatively high mechanical strength, and the only slight and slow solubility of silicates in human body fluid, and so, they can be used as long-term implants [6]. Numerous bioactive silicate glass-ceramics have been developed during the past decades. Glass-ceramics based on silicate, including Bioglass®, glass-ceramics containing the following crystal phases; $Na_2Ca_2Si_3O_9$ as crystal phase [7,8], apatite [9,10], apatite-wollastonite [11,12], apatite-fluorphlogopite [13], apatite-mullite [14,15], fluorrichterite [16–19] and fluorcanasite [20–24] glass-ceramics, are being considered for a wide range of applications, such as middle ear implants, alveolar ridge augmentation, scaffolds for tissue engineering, and the treatment of dentin sensitivity.

Fluorcanasite glass-ceramic ($K_2Na_4Ca_5Si_{12}O_{30}F_4$) which was firstly discovered and developed by Beall in 1980s [25,26] is one of the most attractive glass-ceramic materials due to its high fracture strength and high fracture toughness. These advantageous mechanical properties are due to their microstructure of interlocking laminar crystals [27], which gives these glass-ceramics high flexural strength (> 300 MPa) and high fracture toughness (5 MPa·$m^{0.5}$) [27,28]. This random orientation is attributed to the homogeneous nucleation of CaF_2 crystals in the early stages of the heat treatment process [29].

Several studies evaluated canasite glass-ceramics as biomedical materials for dental and implant applications. Wolcoft reported in his patent the preparation of canasite-apatite glass-ceramics [20]. The final glass-ceramics contained F-canasite as the primary crystal phase and F-apatite as a secondary crystal phase. They possessed a bending strength of > 172 MPa and a fracture toughness of > 2.5 MPa·$m^{0.5}$. Moreover, the biocompatibility and in vitro bioactivity tests of a selected sample showed that the obtained glass ceramic was non-cytotoxic and bioactive. Johnson et al. [21] just investigated the effect of mold and glass casting temperatures on the biaxial flexural strength of the canasite glass-ceramic for dental application, however, they neither evaluated the cytotoxicity nor the bioactivity of the derived glass-ceramic. Additionally, in their later work [30], the effect of using two different furnace types (programmable furnace and dental laboratory burnout furnace) for glass heat-treatment on the biaxial flexural strength was assessed. Likewise, they did not investigate the cytotoxicity and bioactivity of canasite glass-ceramic. Miller et al. [22] followed the formation of apatite layers on canasite glass-ceramics modified by either increasing the concentration of Ca in the glass, or by the addition of P_2O_5 after immersion in simulated body fluid (SBF). Furthermore, they investigated the changes in pH and ions concentrations released from the glass-ceramics into SBF. The study concluded that the increase of the Ca content enhanced the deposition of apatite layer throughout the formation of a silica-rich layer, while, the addition of P_2O_5 led to the deposition of apatite layer directly on the surface without formation of a silica-rich layer due to the existence of fluorapatite crystals in the parent glass-ceramic. Barros et al. [23] investigated in vivo biocompatibility and osteoconductivity of canasite glass ceramics for dental and implant applications. They used hydroxyapatite (HAp) ceramics for comparison. Their study concluded that the investigated canasite glass-ceramics did not exhibit good osteoconductivity unlike HAp, due to its high degradation in vivo, and hence it was not suitable for implant applications. Bandyopadhyay-Ghosh et al. [24] evaluated in vitro biocompatibility of modified fluorcanasite glass-ceramics in comparison with Bioglass®. They modified the phase formation, especially the fluorcanasite concentration by either adding P_2O_5 or changing the Na_2O/CaO ratio. In vitro cell tests were carried out by culturing the samples with osteosarcoma ROS cells for 72 h. Their findings stated that the biocompatibility was sensitive to the glass composition and the concentration of ions released from the samples. Furthermore, the prepared modified fluorcanasite glass-ceramics was reported to possess better in vitro biocompatibility than Bioglass®. Thereafter, they investigated the biocompatibility and osteoconductivity in vivo by implantation in the midshaft of rabbit femora for the same glass-ceramic samples using Bioglass® as a reference [31]. The results showed that the addition of P_2O_5 to the fluorcanasite composition led to a significant enhancement of the osteoconductivity, but there was no evidence for the interface reaction as observed in Bioglass®.

Most of the previous studies were performed after modifying the composition of the canasite glass-ceramics by either adding P_2O_5 or increasing the CaO concentration in the base glass. However, studies on the preparation of glass-ceramics based on canasite and calcium phosphate stoichiometry with different molar ratios were up to now not reported.

The present paper introduces a new bioactive glass-ceramic material based mainly on canasite and calcium phosphate in various molar ratios. Additionally, in vitro bioactivity of the samples was investigated in SBF by following the formation of apatite layer and the concentrations of released ions from the samples into the solution. Furthermore, cytotoxicity tests were performed by culturing the samples with normal human lung fibroblast cells (WI-38 cells) and a baby hamster kidney cell line (BHK cells).

2. Materials and methods

2.1. Glass preparation

Glass-ceramic compositions were prepared with different molar ratios fluorcanasite ($Na_4K_2Ca_5Si_{12}O_{30}F_4$)/tricalcium phosphate ($Ca_3(PO_4)_2$), as shown in Table 1. Glass batches were prepared from reagent grade chemicals of SiO_2 (quartz), CaF_2, Na_2CO_3, $CaCO_3$, K_2CO_3 and $NH_4H_2PO_4$. The batches were mixed for half an hour in an electrical agate ball mill, to ensure a good uniformity and better glass homogeneity. The batches were melted in platinum-10% rhodium crucibles, in an electrical furnace. The furnace temperature was raised slowly and gradually to avoid spattering or splashing of the batch materials during melting. The glass batches were melted in the temperature range from 1250 to 1400 °C, depending on their compositions. The molten batch was held as short as possible at the maximum temperature to avoid excessive fluorine loss. The glass melt was poured into a hot steel mold, and then annealed, at 450 °C in a muffle furnace, to remove strains in the glass samples. Glasses with canasite/tricalcium phosphate molar ratios ≤ 1 devitrified under these conditions, and were excluded from the further study. This was stated if a partial or complete transformation of the transparent glass into translucent or opaque samples with white color was detected.

2.2. Heat-treatment schedules

In order to convert the glasses into glass-ceramics by promoting the process of crystallization, the glass samples were subjected to controlled double-stage heat-treatment schedules according to their DTA profiles. The glass samples were heat-treated up to 550 °C and kept at this temperature for 2 h to ensure sufficient nucleation. After this nucleation treatment, the temperature was raised to the respective crystallization temperature (700, 800, 900 or 1000 °C) and the samples were again kept at this temperature for another 2 h. Accordingly, the samples were encoded as C700, C800, C900 and C1000, beside the as-cast glass was denoted as G sample.

Table 1
Compositions of the investigated glasses in mol%.

Can:TCP molar ratio	SiO_2	Na_2O	K_2O	CaO	CaF_2	P_2O_5	Appearance
Can:TCP = 2	54.55	9.09	4.55	20.45	9.09	2.27	Transparent
Can:TCP = 1	50.00	8.33	4.17	25.00	8.33	4.17	Translucent
Can:TCP = 0.67	46.15	7.69	3.85	28.85	7.69	5.77	Opal
Can:TCP = 0.50	42.86	7.14	3.57	32.14	7.14	7.14	Opal

Can = canasite and TCP = tricalcium phosphate.