Colloids and Surfaces B: Biointerfaces 121 (2014) 82–91

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Osteoblastic differentiation under controlled bioactive ion release by silica and titania doped sodium-free calcium phosphate-based glass

Maziar Shah Mohammadi[a], Florencia Chicatun[a], Christoph Stähli[a], Naser Muja[a], Martin N. Bureau[a,b], Showan N. Nazhat[a,*]

[a] Department of Mining and Materials Engineering, McGill University, Montreal, QC H3A 2B2, Canada
[b] National Research Council Canada, QC J4B 6Y4, Canada

ARTICLE INFO

Article history:
Received 23 January 2014
Received in revised form 30 April 2014
Accepted 27 May 2014
Available online 4 June 2014

Keywords:
Calcium phosphate-based glass
Silica
Osteoinduction
Alkaline phosphatase
Cell–material interaction
Bone repair

ABSTRACT

Sodium-free phosphate-based glasses (PGs) doped with both SiO_2 and TiO_2 ($50P_2O_5$-40CaO-$xSiO_2$-$(10-x)TiO_2$, where x = 10, 7, 5, 3, and 0 mol%) were developed and characterised for controlled ion release applications in bone tissue engineering. Substituting SiO_2 with TiO_2 directly increased PG density and glass transition temperature, indicating a cross-linking effect of Ti on the glass network which was reflected by significantly reduced degradation rates in an aqueous environment. X-ray diffraction confirmed the presence of $Ti(P_2O_7)$ in crystallised TiO_2-containing PGs, and nuclear magnetic resonance showed an increase in Q^1 phosphate species with increasing TiO_2 content. Substitution of SiO_2 with TiO_2 also reduced hydrophilicity and surface energy. In biological assays, MC3T3-E1 pre-osteoblasts effectively adhered to the surface of PG discs and the incorporation of TiO_2, and hence higher stability of the PG network, significantly increased cell viability and metabolic activity indicating the biocompatibility of the PGs. Addition of SiO_2 increased ionic release from the PG, which stimulated alkaline phosphatase (ALP) activity in MC3T3-E1 cells upon ion exposure. The incorporation of 3 mol% TiO_2 was required to stabilise the PG network against unfavourable rapid degradation in aqueous environments. However, ALP activity was greatest in PGs doped with 5–7 mol% SiO_2 due to up-regulation of ionic concentrations. Thus, the properties of PGs can be readily controlled by modifying the extent of Si and Ti doping in order to optimise ion release and osteoblastic differentiation for bone tissue engineering applications.

1. Introduction

Bioactive and bioresorbable bioceramics such as silicate-based glasses (SGs) stimulate osteoblastic osteoinduction responses as they dissolve and are replaced by regenerating bone [1,2]. In particular, ionic products of bioactive glass dissolution including Si^{4+}, P^{5+} and Ca^{2+} have been shown to increase human osteoblast proliferation and protein synthesis [3,4]. Increasing interest in Si-containing bone graft substitutes has also led to the development of Si-substituted calcium phosphates [5,6] including Si-substituted hydroxyapatite or tricalcium phosphate [7]. Similarly, exposure to exogenous P^{5+} and Si^{4+} ions has been shown to increase osteoblastic differentiation at the interface of an Si-rich calcium phosphate where silicon ions up-regulated osteocalcin and osteopontin expression [8]. Silicon also enhances osteoblastic differentiation through increased collagen-I synthesis [9].

Despite the significant interest in Si-substituted calcium phosphates, there is limited evidence of therapeutic release of Si from the calcium phosphate network and it is not known whether the positive biological response of osteoblasts is due to this Si ion release or to a change in material properties; e.g. topographical effects and Ca^{2+} ion release [10]. In addition to silicon, dissolved phosphorous and calcium can either positively or negatively affect the function of osteoblasts depending on their concentration in media. For example, exposure to supplementary Ca^{2+} concentration in the cell culture media has been shown to enhance osteoblastic differentiation [11]. On the other hand, phosphorous ions can stimulate the expression of osteopontin and protein synthesis by osteoblast-like cells, whereby regulation of multiple genes by the increase in phosphate associated with osteoblast differentiation has been revealed [12]. However, elevated P^{5+} concentration has been found to delay osteoblastic differentiation and mineralisation [11,13]. In sum, the controlled release of multiple ionic products by ceramic or glass dissolution exerts complex biological effects on bone–cell function [8].

Phosphate-based glasses (PGs) are soluble due to the hydrolysis of the P—O—P bonds and their solubility and ionic release can

* Corresponding author.
E-mail address: showan.nazhat@mcgill.ca (S.N. Nazhat).

http://dx.doi.org/10.1016/j.colsurfb.2014.05.037
0927-7765/

Table 1
Phosphate glass coding, composition, and processing conditions.

Glass code	Glass composition (mol%)				Processing temperature	
	P_2O_5	CaO	SiO_2	TiO_2	Melting T (°C)/t (h)	Annealing T (°C)/t (h)
Si10	50	40	10	0	1150/1.5	450/2
Si7Ti3	50	40	7	3	1350/3.5	450/2
Si5Ti5	50	40	5	5	1350/3.5	450/2
Si3Ti7	50	40	3	7	1500/3.5	550/2
Ti10	50	40	0	10	1500/3.5	550/2

be controlled through their chemistry, specifically by the addition of modifying oxides [14–16]. Various PG formulations, mainly based on P_2O_5–CaO–Na_2O systems, have been developed and investigated for biomedical applications [17–26]. Na_2O is deemed important to be a major component of the SGs in order to reduce the high network connectivity and increase the surface reactivity [27]. However, PGs are readily soluble in aqueous environments due to their lower network connectivity and may not require Na_2O as a modifying oxide. In addition, Na^+ has been shown to have a deleterious effect (e.g. increased cell death, as well as inhibited cell adhesion and proliferation) on adult human bone marrow stromal cells [28,29]. The addition of CaO, on the other hand, increases the stability of PGs since the divalent cationic nature of Ca ionically cross-links the phosphate chains [30]. High Ca containing metaphosphate glasses have also been shown to increase cellular proliferation compared to PGs doped with lower calcium content [17].

The incorporation of titania (TiO_2) into PGs has been investigated to reduce their degradation rate and improve their long-term biological effects [19,31–34]. A reduction in degradation rate was obtained by incorporation of up to 5 mol% TiO_2 into P_2O_5-CaO-Na_2O system, which was attributed to the formation of strong P—O—Ti bonds. Moreover, addition of TiO_2 enhanced the attachment and viability of MG63 human osteosarcoma cells [19]. Titanium PG microspheres in the $50P_2O_5$-40CaO-$(10-x)Na_2O$-$xTiO_2$ system have shown significant densification of the PG structure upon TiO_2 incorporation up to 5 mol%; whereas, further TiO_2 incorporation up to 7 mol% was not effective to the same extent [35]. Small variation in TiO_2 content significantly reduced the PG dissolution rate in the $50P_2O_5$-30CaO-$9Na_2O$-$3SiO_2$-$3MgO(5-x)K_2O$-$xTiO_2$ system [36]. In contrast, the incorporation of SiO_2 into PGs increases the degradation rate by disrupting the glass network [14,37]. Therefore, because bonds formed in SiO_2-P_2O_5 network may be more sensitive to hydrolytic activity [14], Si substitution may be a suitable alternative to Na_2O containing PGs for the controlled release of bioinorganics for bone repair.

In this study, sodium-free PGs doped with different proportions of silica and titania were developed to assess the influence of Si and Ti on the properties of bioinorganic releasing glasses. The physicochemical, dissolution, ion release and surface characteristics of these PGs were investigated and the effect of their ionic release by PGs on the viability and osteogenic differentiation of MC3T3 preosteoblasts were investigated. In sum, the properties of PGs can be tailored for bone tissue engineering applications by incorporating silica and titania into the PG formulation.

2. Materials and methods

2.1. PG production

P_2O_5, $CaHPO_4$, SiO_2 (Alfa Aesar, Canada), and TiO_2 (Sigma Aldrich, Canada) precursors were used to produce the melt derived PG compositions given in Table 1. The precursors were dry blended, poured into a Pt/10%Rh crucible (Kitco Inc.) and placed into a furnace (Carbolite RHF1500, Ancansco) at 350 °C for 20 min in order to remove the moisture. The precursors were then melted at various temperatures and times as determined by their glass composition (Table 1). The melt was cast in a pre-heated cylindrically shaped graphite mould (10 mm in diameter), and annealed to remove the residual stresses (Table 1). The glass rods were then cut into approximately 2 mm thick discs using a diamond blade cutting machine (Buehler, IsoMet®).

2.2. Characterisation of the bulk and surface properties of PGs

2.2.1. Density measurements

Density measurements were carried out using Archimedes' principle ($n = 3$) through a density kit attached to an analytical balance (Mettler Toledo, AB265-S/FACT). Ethanol was used as the submersion liquid.

2.2.2. Differential thermal analysis

Differential thermal analysis (DTA, TA Instruments SDT Q600) was conducted to identify the three main thermal parameters: glass transition temperature (T_g), crystallisation temperature (T_c) and melting temperature (T_m). The test was carried out on powdered glass samples of approximately 50 mg from 25 °C up to 1200 °C at a heating rate of 10 °C/min under nitrogen purge. An empty crucible was used as a reference.

2.2.3. X-ray diffraction of the crystallised PG

X-ray diffraction (XRD) was used to identify the phases that are present in the crystalline state of the glasses. The glass compositions were crystallised at the temperatures obtained from DTA for 3 h. The test was conducted on a Bruker Diffractometer (Bruker AXS Inc.) in flat plate geometry from $2\theta = 6$–$86°$, with Ni-filtered Cu K_α radiation, and a Philips (PW1710) from $2\theta = 5$–$100°$ with a step size of 0.02 and a count time of 0.1 s. The patterns were analysed using the EVA software package.

2.2.4. Nuclear magnetic resonance (NMR)

^{31}P MAS NMR spectra were obtained at a frequency of 161.8 MHz on a Varian VNMRS400 spectrometer. Samples were placed in 4 mm rotors and spun at 12,500 Hz. A 30° pulse was applied every 5 s and 128 scans were accumulated. Spinworks software was used for deconvolution of the spectra and the calculation of the relative fractions of different phosphate species in the PG network.

2.2.5. Wettability and surface free energy of PGs

Glass discs from each PG composition were abraded against silicon carbide paper (1200/4000, Struers) using absolute ethanol as the lubricant. Prior to the test, the samples were ultrasonicated in ethanol for 20 min and allowed to dry. The static contact angle was measured with a contact angle system OCA (Future Digital Scientific) and ultrapure water (UW) and diiodomethane (DII) were used to represent both polar and non-polar characteristics, respectively. Droplets (5 μL) were placed on the glass surface via a syringe and the drop profile was recorded for 10 s. The contact angle was calculated after 2 s from the time the droplet was in contact with the surface. The measurements were carried out in triplicate. The surface free energy was calculated using the Owens, Wendt, Rable and Kaelble (OWRK) method via SCA20 software.

2.3. Ageing of PGs in deionised water

PG discs ($\Phi = 10$ mm, $n = 3$) were placed in polypropylene vials containing 10 ml of ultra-pure deionised water (DW, 18.2 MΩ cm resistivity) and incubated for up to 28 days at 37 °C. Ion release, weight loss, and pH of the ageing environment were measured at 6, 24, 72, 168, 336, 504 and 672 h.