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Titanium phosphate glass microspheres for bone tissue engineering

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ABSTRACT

We have demonstrated the successful production of titanium phosphate glass microspheres in the size range of ~10–200 μm using an inexpensive, efficient, easily scalable process and assessed their use in bone tissue engineering applications. Glasses of the following compositions were prepared by melt-quench techniques: $0.5P_2O_5$–0.4CaO–$(0.1-x)Na_2O$–$xTiO_2$, where $x = 0.03$, 0.05 and 0.07 mol fraction (denoted as Ti3, Ti5 and Ti7 respectively). Several characterization studies such as differential thermal analysis, degradation (performed using a novel time lapse imaging technique) and pH and ion release measurements revealed significant densification of the glass structure with increased incorporation of TiO_2 in the glass from 3 to 5 mol.%, although further TiO_2 incorporation up to 7 mol.% did not affect the glass structure to the same extent. Cell culture studies performed using MG63 cells over a 7-day period clearly showed the ability of the microspheres to provide a stable surface for cell attachment, growth and proliferation. Taken together, the results confirm that 5 mol.% TiO_2 glass microspheres, on account of their relative ease of preparation and favourable biocompatibility, are worthy candidates for use as substrate materials in bone tissue engineering applications.

1. Introduction

Titanium phosphate glasses have been widely researched for use in orthopaedic applications because of their highly favourable material properties and ability to elicit a positive bone cell response [1–4]. From a materials science perspective, these glasses are of great interest because their physicochemical properties are highly tuneable, so that subtle changes in glass composition allow for major changes in glass structure and consequently in the degradation and ion release behaviour of these materials [5,6]. Changes in titanium phosphate glass chemistry can be brought about by variations in the concentrations of the constituent oxides or the addition of small amounts of metal oxides other than TiO_2 [7–13]. The structure of the glass network has been elucidated using diverse analytical techniques such as differential thermal analysis (DTA), Raman and Fourier transform infrared (FTIR) spectroscopies, X-ray diffraction (XRD), solid state nuclear magnetic resonance (NMR), X-ray absorption spectroscopy (XAS), Ti K-edge X-ray absorption near-edge structure (XANES) and neutron/X-ray scattering [6]. The information gained from all these analyses is beneficial to biomaterials researchers, who are then able to make highly informed choices regarding the specific glass composition required for specific clinical applications.

From a biological perspective, it is now well known that titanium phosphate glasses provide a surface that is quite conducive to the attachment, growth and proliferation of bone cells [14–16]. The fact that oxides of phosphorus, sodium and calcium, which constitute the major components of most phosphate glass compositions, are also found in the mineral phase of bone is a major contributing factor to the bioactivity of these glasses, particularly considering that the ions released from these glasses can exert positive effects on bone cells [17–20]. A range of *in vitro* and *in vivo* studies has been conducted on titanium phosphate glasses and highly promising results have been obtained that offer exciting

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possibilities for using titanium phosphate glasses in various therapeutic applications to combat bone loss [8,13,14,21–23].

In order to realize viable commercial applications of these glasses, some form of glass processing is essential, for which it is necessary to develop glass processing methodologies that are efficient, cost-effective and easily scalable. However, relatively little research has been published thus far on processing routes that would be suitable for titanium phosphate glasses. To some extent, this can be explained by the difficulties involved in processing these glasses. Glass microfibres have been commonly produced by drawing glass melt onto a rotating steel drum at high temperature. This method has been used to produce iron phosphate glass fibres that have demonstrated their efficacy in the tissue engineering of muscle cells and neuronal cells [24–27]. However, only one report in the literature exists on the use of this method to produce titanium phosphate glass fibres [28].

In this study, we investigate the production of microspheres of melt-derived phosphate glasses using a flame spheroidization technique. The rationale for processing titanium phosphate glasses in microsphere form (as opposed to using crushed glass particles of random shapes) is two-fold. First, from a commercial standpoint, the spherical shape of these particles means that the surface area and properties (e.g. degradation behaviour and ion release in various media) of individual particles and consequently of a large batch of microspheres (possibly in the kilogram range) can be easily quantified. Second, from the standpoint of cell growth and proliferation in dynamic three-dimensional environments, microspheres would provide larger interstitial spaces that are more consistent and quantifiable for cell growth and proliferation than random-shaped particles when packed into systems such as perfusion bioreactors. The main reason for using the flame spheroidization technique is that microspheres can be produced very quickly since the particle residence time in the flame is of the order of milliseconds, which makes this method feasible for commercial synthesis of titanium phosphate glass microspheres for use in biomedicine. The major application envisaged for these microspheres is as microcarriers for scale-up of different cell types, including bone cells, in bioreactors. The large quantities of cells thus produced can be used for regenerative cell therapy applications as well as for high-throughput screening.

2. Materials and methods

2.1. Glass manufacture

The glasses (a total of three compositions; see Table 1) were manufactured according to the methods of Abou Neel et al. [7] using stoichiometric quantities of the following precursors (all with purities of >98% and obtained from VWR-BDH, Poole, UK) without further purification: phosphorus pentoxide (P_2O_5), calcium carbonate ($CaCO_3$), sodium dihydrogen orthophosphate (NaH_2PO_4) and titanium oxide (TiO_2). After preheating at 700 °C for 30 min to remove CO_2 and H_2O, the precursor mixture was then melted according to the conditions listed in Table 1. After melting for the required period, the glass was rapidly quenched by pouring onto a steel plate at room temperature and then allowed to cool overnight.

2.2. Preparation of glass microspheres

The glasses obtained from the melt quench step were broken into fragments and the fragments were crushed to form microparticles using a Retsch MM301 milling machine operated at a frequency of 10 Hz (Retsch, Germany). The microparticles were then passed through a flame spheroidization apparatus to produce microspheres in the approximate size range of 10–200 μm.

The flame spheroidization apparatus comprises the following component assemblies: (1) blow torch, (2) feed and (3) collectors (see Fig. 1). The blow torch assembly consists of a Rothenberger Super Fire 2 gas torch (Rothenberger Werkzeuge GmbH, Kelkheim, Germany) fitted to a 453 g yellow MAP-PLUS gas cylinder (Today's Tools, UK). MAP-PLUS gas is a generic substitute for the trademarked MAPP gas (methylacetylene-propadiene propane) that has a high flame temperature of 2925 °C in oxygen. The blow torch assembly is positioned such that the flame of the gas torch is horizontal. The feed assembly comprises an aluminium trough with dimensions of 200 × 20 × 30 mm, with the edge of one end positioned ~10 mm above the outlet of torch at a slight angle to the horizontal. A DC motor (15800 rpm, 4.5–15 V, 35.8 mm diameter; RS components, Corby, UK) is attached to the other end of the trough and is connected to an ISO-TECH IPS-405 programmable power supply (RS Components, Corby, UK). A metal screw connected to the axle of the motor serves as an offset to generate vibration when the motor is in operation so that the particle feed for microsphere production is distributed over the surface of the trough before the particles enter the flame. For optimum particle distribution in the trough during normal operation, the output power from the programmable power supply is maintained at 4 W. The collectors consist of four rectangular glass boxes with dimensions of 275 × 150 × 60 mm placed with their longer edges in contact with each other. The first collector is placed directly below the flame so as to collect particles that do not pass through the flame.

During operation, glass microparticles placed at one end of the trough travel to the other end under the influence of vibratory forces exerted by the DC motor. At the other end, the particles pass into the flame and then travel along the flame axis. As they pass through the flame, they undergo spheroidization due to surface tension forces and are then collected in the glass boxes placed one after the other below the flame (Fig. 1).

The microspheres from each collector were separately visualized using light microscopy. The material present in the collector immediately below the torch outlet was discarded since it usually contained a mixture of glass microspheres along with a significant proportion of non-spherical particles; the material in the remaining glass boxes contained very few non-spherical particles, if any, and was therefore collected and stored for further studies.

Table 1
List of compositions, codes used and thermal and structural properties.

Glass code	Glass composition (mol.%)				Melting temperature (°C)	Melting time (h)	Density (g cm^{-3})	T_g (°C)	T_c (°C)	T_m (°C)	Q^1* chemical shift (ppm)	Q^2 chemical shift (ppm)	Q^1** (%)	Q^2 (%)
	P_2O_5	CaO	Na_2O	TiO_2										
Ti3	50	40	7	3	1300	3	2.635 ± 0.004	489	689	878	−9.4	−26.3	0.3	99.7
Ti5	50	40	5	5	1300	3	2.653 ± 0.001	510	720	904	−9.6	−26.5	0.5	99.5
Ti7	50	40	3	7	1350	5.5	2.671 ± 0.002	529	739	905	−10.1	−26.8	1.1	98.9

* Errors in Q^1 and Q^2 chemical shifts are 0.5 ppm for all compositions.
** Errors in Q^1 and Q^2 percentage values are 1% for all compositions.