Materials Letters 214 (2018) 268–271

Contents lists available at ScienceDirect

Materials Letters

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

Nanostructured apatite-mullite glass-ceramics for enhanced primary human osteoblast cell response

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ARTICLE INFO

Article history:
Received 14 August 2017
Received in revised form 27 November 2017
Accepted 10 December 2017
Available online 11 December 2017

Keywords:
Glass-ceramic
Bioceramics
Nanocrystalline materials
Cell response

ABSTRACT

This work investigates the difference in viability of primary human foetal osteoblast cells on a glass-ceramic surface with nanoscale topography relative to viability on a smooth glass-ceramic surface containing a bioactive phase. Apatite-mullite glass-ceramics containing bioactive fluorapatite ($Ca_{10}(PO_4)_6F_2$) and bioinert mullite ($Si_2Al_6O_{13}$) were synthesised and subsequent heat-treatment was optimised to form nano-sized fluorapatite crystals. Etching was used to selectively remove the bioactive phase, producing a surface with disordered nanoscale topography. Cells were seeded onto a smooth polished glass-ceramic substrate with the bioactive phase intact, an etched nanostructured glass-ceramic with the bioactive phase removed, and a borosilicate glass control. Cell viability after 24 h and 48 h was significantly greater on the nanostructured surface compared to the smooth bioactive surface, while cell viability at both time points was significantly greater on both nanostructured and smooth bioactive surfaces compared to the control.

1. Introduction

The fixation of orthopaedic implants to surrounding tissue can be greatly improved by promotion of osteointegration [1]. The surface chemistry of implants has been shown in many studies to influence osteointegration [2,3], and bioactive surface coatings such as hydroxyapatite are therefore commonly applied [3]. Apatite-mullite glass-ceramics (AMGCs) are being investigated as an alternative surface coating [4]. AMGCs are triphasic materials composed of fluorapatite ($Ca_{10}(PO_4)_6F_2$) and mullite ($Si_2Al_6O_{13}$) in a residual glass matrix [5]. Fluorapatite is bioactive and promotes cellular attachment [6,7] and the presence of fluoride ions promotes bone regeneration [8].

Surface topography at the nanoscale has been shown in many studies to significantly influence osteoblast attachment, adhesion, proliferation, and differentiation on a range of biomaterials [9–12]. Nanoscale disordered topographies using polymethylmethacrylate (PMMA) substrates have been shown by Dalby et al. to stimulate mesenchymal stem cells (MSCs) to proliferate, differentiate and produce more bone mineral than MSCs seeded onto smooth PMMA substrates [11], demonstrating that surface topography can have significant biological effects.

The crystallisation [13] of AMGCs can be controlled such that only nano-sized fluorapatite crystals are formed. Such AMGCs offer the potential to create nanoscale topography similar to those presented by Dalby et al. [11] by selective etching of the fluorapatite phase at the glass-ceramic surface [14]. However, given that fluorapatite is the phase that is typically etched, the topography is produced by removing the bioactive phase from the surface. This provides a novel opportunity to compare the influences of surface chemistry and surface topography on cell viability: the influence surface chemistry can be examined by seeding cells onto an AMGC with the bioactive phase present, with the influence of topography examined by seeding cells onto a disordered nanoscale topography created by removing the bioactive phase.

Immortalised cell lines are widely used in cell culture studies [15]. However, here, for better clinical relevance, primary cells are used.

2. Materials and methods

A glass with molar composition $4.85SiO_2$–$3.25Al_2O_3$–$1.5P_2O_5$–$3.25CaO$–$1.75CaF_2$, chosen following a review of previous studies [14,16,17], was synthesised by melt-quench, as previously described [5,14]. This composition may produce crystal sizes and spatial density similar to the topographies used by Dalby et al.

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https://doi.org/10.1016/j.matlet.2017.12.051
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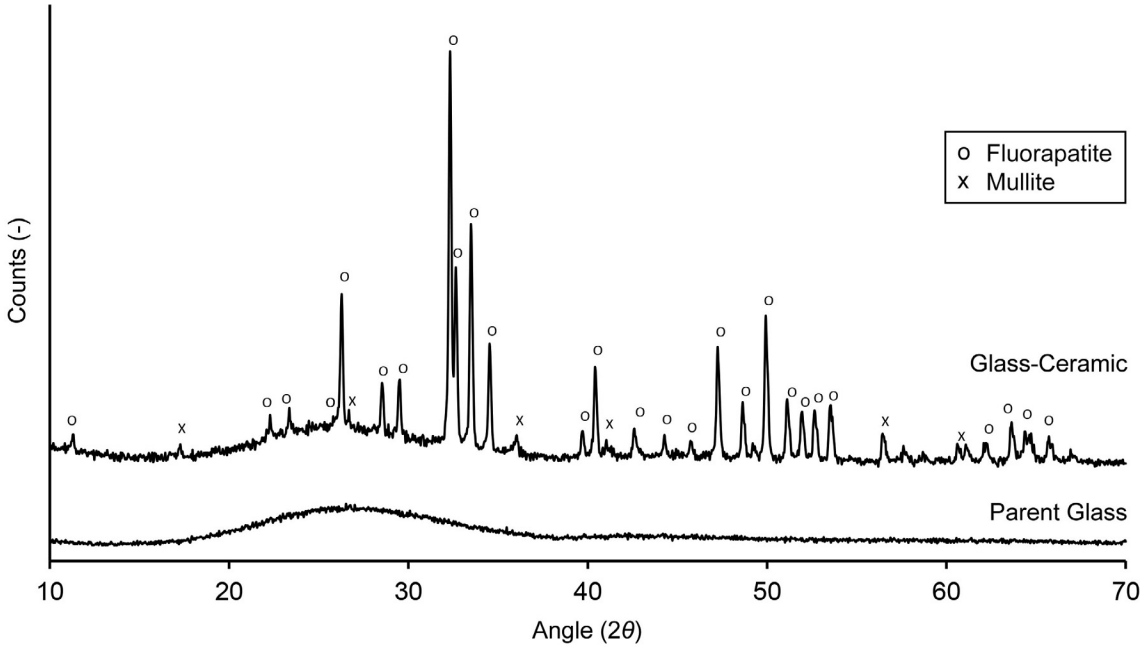

Fig. 1. XRD profiles for as-cast glass and AMGC heat-treated at T_{p2}.

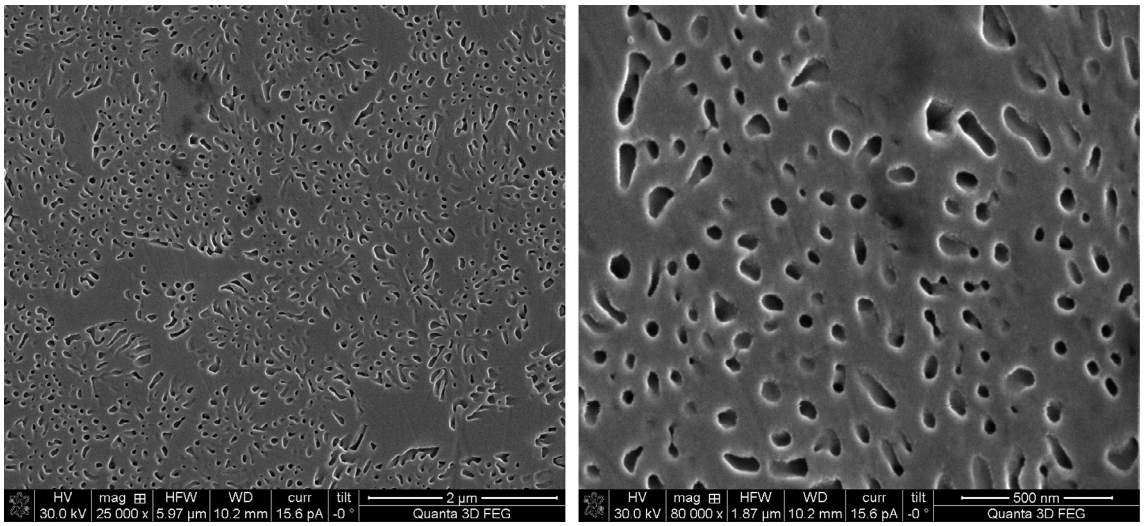

Fig. 2. Micrographs of AMGC heated to 1100 °C for 20 min and etched.

[11]. Some of the glass was re-melted and cast to form cylinders (Ø 11 mm) with annealing at $T_g - 100$ °C (560 °C) [14]. Cast cylinders were sectioned into 2.5 mm thick discs.

Glass frit was characterised by differential scanning calorimetry (DSC) [14] to determine the glass transition temperature (T_g) and primary and secondary crystallization peak temperatures (T_{p1} and T_{p2}).

Glass-ceramics were formed by heating glass discs at 10 °C/min to a range of temperatures above T_{p2}, with dwell periods of 0–60 min, followed by cooling in air. Samples were then ground and polished to a sub-micron finish [16].

X-ray diffraction (XRD) was carried out to confirm the amorphous nature of cast glass samples and identify the phases present in heat-treated samples using a Siemens D500 diffractometer (Munich, Germany) with CuKα X-rays from 10–70° 2θ. Phases were identified using JCPDS-ICDD PDF cards 15-876 (fluorapatite) and 15-776 (mullite).

Glass-ceramic samples were etched for 20 s in 1 M HNO_3, followed by rinsing in water followed by ethanol and drying. This removes the fluorapatite phase from the surface, leaving the bioinert mullite phase and residual glass.

Microscopical analysis was performed to identify the ideal crystallisation temperature and dwell period for formation a nanostructured surface. Samples were gold sputter coated and imaged using an FEI Quanta 3D FEG-SEM (Eindhoven, The Netherlands). The average pore size and spatial density of pores on substrate surfaces were determined using ImageJ [18].

Three different substrate types were used for cell culturing: a smooth polished AMGC, an etched AMGC, and a borosilicate glass control.

Glass-ceramic samples for cell study were prepared by heat-treating using the ideal crystallisation temperature and dwell period, as determined by microscopical analysis. For sterilisation prior