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Biological evaluation of an apatite–mullite glass-ceramic produced via selective laser sintering

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Abstract

The biological performance of a porous apatite–mullite glass-ceramic, manufactured via a selective laser sintering (SLS) method, was evaluated to determine its potential as a bone replacement material. Direct contact and extract assays were used to assess the cytotoxicity of the material. A pilot animal study, implanting the material into rabbit tibiae for 4 weeks, was also carried out to assess in vivo bioactivity. The material produced by SLS did not show any acute cytotoxic effects by either contact or extract methods. There was no evidence of an apatite layer forming on the surface of the material when soaked in SBF for 30 days, suggesting that the material was unlikely to exhibit bioactive behaviour in vivo. It is hypothesized that the material was unable to form an apatite layer in SBF due to the fact that this glass-ceramic was highly crystalline and the fluorapatite crystal phase was relatively stable in SBF, as were the two aluminosilicate crystal phases. There was thus no release of calcium and phosphorus and no formation of silanol groups to trigger apatite deposition from solution within the test time period. Following implantation in rabbit tibiae for 4 weeks, bone was seen to have grown into the porous structure of the laser-sintered parts, and appeared to be very close to, or directly contacting, the material surface. This result may reflect the local environment in vivo compared to that artificially found with the in vitro SBF test and, furthermore, confirms previous in vivo data on these glass-ceramics.

Keywords: Glass-ceramic; Bioactivity; Layer manufacturing; Bone ingrowth; Bone replacement

1. Introduction

Solid freeform fabrication (SFF) technology is increasingly being recognized for its potential to produce implants with predefined and reproducible external and internal morphology that could be used in bone replacement applications. This series of manufacturing techniques build objects additively, on a layer-by-layer basis, directly from a three-dimensional, computer-designed representation of the part, without the need for specialized moulds, dies or tooling [1,2]. They are able to rapidly and reproducibly produce highly complex three-dimensional physical objects, and thus offer the potential to provide a cost-effective, efficient method by which to construct implants. The utilization of computer-aided design (CAD) models as inputs for the fabrication of parts means that patient-specific data and internal structural features can be incorporated into the design of the implants. A controlled internal structure of interconnected porous channels is desired so as to provide a framework for bone to grow into the matrix of the material, increasing the interfacial area between the implant and the tissue, and thereby reducing the movement of the implant in the tissue [3]. Suitable channels must also be present so as to allow a blood supply to reach the connective ingrown tissue, thereby preventing necrosis of the penetrating cells.

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We have previously demonstrated that it is possible to use one of these layer technologies, selective laser sintering (SLS), which builds parts by selectively fusing successive thin layers of powdered material, to produce porous parts from glass-ceramic materials [4]. The study used a glass based on the system SiO_2–Al_2O_3–P_2O_5–CaO–CaF_2, which crystallizes to a glass-ceramic with apatite and mullite phases (A–M glass-ceramic) and has been shown to produce a material with good fracture toughness and mechanical strength [5]. An in vivo study by Freeman et al. demonstrated that whilst the parent glass was encapsulated by fibrous tissue, following crystallization, A–M glass-ceramics showed evidence of osseointegration and osteoconduction with new bone in contact with the implant material [6].

An indirect SLS method was used, mixing the starting glass powder with an acrylic binder before laser sintering to produce a green part. The material then required post-processing to burn off this binder and crystallize out the apatite and mullite phases. Although the main phases were apatite and mullite, a third phase, anorthite, was also present. This is known to form in these materials when there is a large surface area, as is found in the laser-sintered samples, due to fluorine release. Parts with good structural integrity and flexural strengths of around 16 MPa, similar to that of cancellous bone, were achieved [4]. The aim of the current study was to carry out a biological evaluation of the glass-ceramic parts produced by this method through a combination of cytotoxicity, and in vitro and in vivo bioactivity testing.

2. Materials and methods

2.1. Production of A–M glass-ceramic by indirect selective laser sintering

A castable glass with the composition $4.5SiO_2$–$3Al_2O_3$–$1.6P_2O_5$–3CaO–$2CaF_2$ (molar ratio) was prepared by a conventional melt-quenching technique. Glass powder with a particle size range of 45–90 μm was mixed with a commercially available acrylic binder (DTM Corporation, now 3D Systems) at 5 wt.% in a rotary mill for 1 h. The glass/binder mix was laser sintered on an experimental machine at the University of Leeds, UK which comprised two 125 W CO_2 lasers combined to produce a total power output of 250 W. A laser scan speed of 250 mm s^{-1}, laser power of 100 W, scan line overlap of half the 1.1 mm beam diameter, and a spread layer depth of 0.25 mm were used to produce the green parts. The samples were post-processed by placing them in a furnace preheated to 1200 °C for 1 h.

2.2. Production of materials to be used as controls

Previous assessment of the bioactivity of a similar A–M glass-ceramic has been performed on samples produced by conventional casting [6]. Therefore it was considered necessary to test cast samples of this A–M glass-ceramic in addition to those produced by SLS, in order to determine whether laser sintering or the subsequent post-processing route altered the bioactivity of the parts. The cast A–M glass-ceramic controls were produced after the method of Lorrison et al. [7]. It should be noted that these samples were considerably more dense than those produced by SLS.

A known bioactive glass similar to Bioglass®, 45S5 [15], and a non-bioactive commercial silica glass were also used as controls for the in vitro bioactivity study, and are referred to hereafter as the PC (positive control) glass and the NC (negative control) glass, respectively. The PC glass, with composition $46.1SiO_2$–$24.4Na_2O$–26.9CaO–$2.6P_2O_5$ (molar ratio) was prepared from reagent grade Na_2CO_3, $CaCO_3$, $Ca_2P_2O_7$ and SiO_2 using a conventional melt-quenching technique. A commercial glass supplied by Matsunami Glass Ind. Ltd., Japan with a composition (wt.%) of $72.6SiO_2$–$1.8Al_2O_3$–3.8MgO–7.9CaO–$12.1Na_2O$–0.1Fe was used as the NC glass.

A porous A–W glass-ceramic, commercially produced under the trade name Cerabone® (Nippon Electric Glass Co, Ltd., Japan), was used as a control for the in vivo study. For this purpose, it was formed into cylindrical samples of approximately 4 mm in diameter and 10 mm in length using grinding paper.

2.3. Cytotoxicity studies

Discs of A–M glass-ceramic, 10 mm in diameter and 2 mm thick, were produced by indirect SLS. Cast A–M samples, with similar dimensions, acted as controls. Assessments of cytotoxicity were carried out according to ISO 10993-5 [8] using both extract and direct contact methods.

The extract assay used normal human dermal fibroblasts (HDFs) and an immortalized human osteoblastic cell line, MG-63, as test cells. Following sterilization by autoclaving at 121 °C and 15 bar for 20 min, the A–M samples were immersed in Dulbecco's modified Eagle's medium (DMEM) at a ratio of 10 ml of medium to 1 g of ceramic. Gentle agitation at 37 °C for 24 h was used to extract the samples, after which the extract solution was collected and stored frozen at −80 °C. Fresh medium was added and the samples incubated under the same conditions for a further 144 h. Again, after the incubation the extract solution was collected and stored frozen. Negative controls were prepared by incubating extracting solution alone for the equivalent times. Two microculture assay plates were prepared by standard procedures – one containing human dermal fibroblasts (HDFs) and one containing MG-63 cells. The extract solutions were thawed and prepared into full culture medium by the addition of 10% (v/v) foetal calf serum, 2 mM L-glutamine, 100 μg ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin. A positive control of full medium containing 0.015% sodium dodecyl sulphate was also assessed. The extract solutions were added to the assay plates in quadruplicate samples and the plates incubated at 37 °C for 24 h. The solutions were then removed and a MTT viability assay run on the plates according to standard procedures [9].

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