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In vitro study of hydroxyapatite-based photocurable polymer composites prepared by laser stereolithography and supercritical fluid extraction

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Abstract

The fabrication of three-dimensional (3-D) structures using computer-controlled ultraviolet (UV) photopolymerization of acrylates (laser stereolithography) often results in the trapping of residual unreacted monomer and initiator. These residuals can leach from the finished structure and affect the biological response of cells and tissues. Thus the potential applications of these structures for tissue engineering have not been fully realized. In this paper we demonstrate that conventional post-lithography treatments followed by processing in the environmentally benign solvent, supercritical carbon dioxide ($scCO_2$), dramatically increased biocompatibility. The $scCO_2$ processing of pure polyacrylate and polyacrylate/hydroxyapatite composite structures extracts residuals from all structures including those that had received full conventional post-lithography treatment (acetone washing/UV drying). Human osteoblast cells seeded on the extracted surfaces of these structures demonstrated increased cell attachment and proliferation on the $scCO_2$-treated materials.

Keywords: Hydroxyapatite; Supercritical CO_2; Laser stereolithography; Biocompatibility; Osteoblast

1. Introduction

Three-dimensional (3-D) scaffolds are used in tissue engineering strategies to provide volume fill, mechanical integrity and a surface that can present chemical and architectural guidance for regenerating tissues [1]. Conventionally these structures have been fabricated by fibre bonding, melt extrusion, solvent casting, particulate leaching and dense gas processing. A technique developed over recent years, rapid prototyping, allows the fabrication of structures for which external shape and internal architecture can be precisely controlled. This is a distinct advantage over conventional methods.

Rapid prototyping (RP) – also called solid freeform fabrication – is a collective name used to describe a number of automated techniques that use computer-controlled equipment to repetitively deposit and process material layers [2,3]. The method relies on computer-aided design to create an image of a structure. This is then sent to the computer that controls the deposition, allowing the image to be turned into a 3-D structure.

Laser stereolithography is one of the most highly developed RP techniques and is based upon ultraviolet (UV) laser-induced photopolymerization of photocurable resins [4]. This technology and commercially available equipment are widely used to fabricate 3-D polymeric biomodels using computer tomography or magnetic reso-

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nance imaging data [5,6]. These biomodels can be used not only for better diagnosis and surgical rehearsal, but also to make moulds for real implant manufacture from biocompatible materials. In recent years, the laser stereolithography technique has started to be used directly for custom-designed implants or scaffolds for tissue engineering. However, this is limited because only a few special acrylic and epoxy-based monomers have the appropriate characteristics to allow effective UV laser-induced photopolymerization to take place. Poly(propylene) fumerate is one of the few polymers that has been successfully optimized for *in vivo* biomedical applications [7].

Perhaps the greatest difficulty with using acrylic polymer objects is contamination due to residual unreacted monomers, low-molecular-weight oligomers, initiators and binding agents. These contaminants can leach from the finished structure and dramatically affect the biological response of cells and tissues. *In vivo* leachates from acrylate polymers can accelerate extracellular matrix degradative pathways and inhibit matrix synthesis [8,9]. They can also lead to various inflammatory and dystrophic processes [10,11]. These factors mitigate against the use of laser stereolithography for the development of scaffolds for tissue engineering and guided bone regeneration.

Previously we have demonstrated that laser-induced photopolymerization of a liquid mixture of polyfunctional acrylic monomers (oligocarbonate dimethacrylate, OCM-2™) and photoinitiator can be used to fabricate scaffolds for *in vivo* bone regeneration [12]. There were two key changes to the procedure that allowed the fabrication of scaffolds for *in vivo* use. The first of these was the incorporation of hydroxyapatite (HA – $Ca_{10}(PO_4)_6(OH)_2$) into the acrylic polymer. HA is a material analogous to the main mineral component of human bone. Its surface is highly bioactive and can bond strongly with the host bony tissue [13]. The presence of HA in a material can change the tissue response to that material, leading to favourable bone apposition rather than fibrous encapsulation [14]. The second notable change to the procedure was a post-fabrication process that extracted residual monomer from the 3-D structure. The extraction was achieved using carbon dioxide gas. Carbon dioxide above its critical temperature and pressure (i.e. 304.1 K and 73.8 bar pressure) is said to be supercritical ($scCO_2$) [15]. $ScCO_2$ is a unique processing medium as it behaves as both a gas and a liquid, which allows it to diffuse into polymers, dissolve substances in the material and thereby extract from the material unwanted impurities [12,16,17].

In this paper human osteoblast (HOBS) cell attachment on OCM-2 polymer and polymer composite structures is investigated. Cell attachment was investigated with respect to conventional post-lithographic treatments (acetone washing, UV drying) and/or $scCO_2$ extraction of the 3-D structures, to examine changes in the biocompatibility of the material [18].

2. Materials and methods

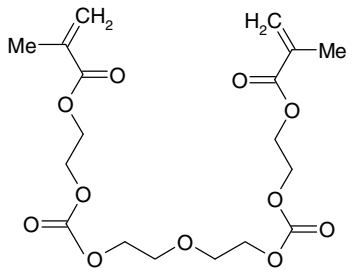
2.1. Materials and composite fabrication

OCM-2 (Fig. 1) was synthesized at the Institute of Chemical Physics (Moscow, Russia) and was used as received. Irgacure 671™ (2,2-dimethoxy-2-phenylacetophenone) was utilized as a photoinitiator for radical polymerization. To prevent spontaneous polymerization, bis-(5-methyl-3-*tert*-butyl-2-oxyphenyl)-methane was used as an inhibitor. Hydroxypol™, a monodisperse hydroxyapatite powder (Polystom Ltd., Moscow, Russia), was used as both bioactive and reinforcing mineral filler in the resins.

The laser stereolithography apparatus (LS-250), designed and produced at the Institute of Laser and Information Technologies (Shatura, Russia), was used to prepare pure polymer and polymer composite structures (Fig. 3). Full details of the experimental procedure have previously been reported [12]. The design of the structures consists of two parts: a solid stub at the base of the structure, which is used predominately for handling and manipulation, and an upper section consisting of alternating lattices and columns (Fig. 3). Each cylindrical structure had a diameter of 4 mm and height of 5 mm. Each structure was produced with an accuracy of 0.1–0.2 mm and the geometry of the samples was controlled and analysed by direct measurements with a micrometer. The porosity of the upper section was calculated from the ratio of the total volume of space/total volume of sample and resulted in a porosity of 40%. For the composites, a homogeneous mixture of HA particles (ca. 1 μm, 30 wt.%) and monomer liquid was prepared by agitation, prior to laser photopolymerization.

2.2. Post-lithography treatment

Following the stereolithography fabrication of the polymer and composite structures, the samples were either left untreated or treated and fixed by conventional post-lithography treatments (i.e. an acetone wash to remove non-processed liquid monomer and/or UV drying to cross-link and fix the structure). The treatments resulted in six test groups



$[CH_2 = C(CH_3)COOCH_2CH_2OCOO]_2R$

R=(-$CH_2CH_2OCH_2CH_2$-)

Fig. 1. Chemical structure of oligocarbonate dimethacrylate (OCM-2™).

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