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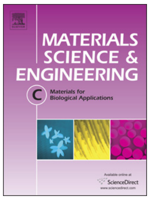

Photocurable high internal phase emulsions (HIPEs) containing hydroxyapatite for additive manufacture of tissue engineering scaffolds with multi-scale porosity

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ABSTRACT

Porous composites containing hydroxyapatite (HA) were templated from high internal phase emulsions (HIPEs) and were further structured using direct-write UV stereolithography to produce composite scaffolds with multi-scale porosity. FTIR, TGA and SEM analyses confirmed that HA was retained after photocuring and subsequent treatments and was incorporated within the polymerised HIPE (polyHIPE). The addition of HA particles to the polyHIPE caused changes in the mechanical properties of the material. An increase in both the Young's modulus and maximum stress at yield was observed compared with the pure polyHIPE from 1.544 ± 0.231 to 4.614 ± 0.775 and 0.177 ± 0.009 to 0.267 ± 0.034 MPa, respectively. Except at very high concentrations, adding HA did not adversely cause the phase separation of the HIPE or the porous microstructure of the resulting polyHIPE. In combination with a photoinitiator, the HIPE emulsion containing HA was investigated as a photocurable resin for stereolithography-based additive manufacturing. The material was readily processable into "woodpile" structures via direct-write UV stereolithography, producing scaffolds with multi-scale porosity which may be useful for medical applications such as tissue engineering. In conclusion, HA was successfully added into polyHIPEs, producing a similar porous structure to that of the pure polyHIPE whilst improving the mechanical performance.

1. Introduction

Synthetic polymers, when used for tissue engineering scaffolds, must be prepared with a porous microstructure. An interesting route for introducing microporosity within a scaffold is emulsion templating using high internal phase emulsions (HIPEs) in which the continuous phase contains monomer and crosslinker and the internal (often aqueous) phase constitutes the majority of the total volume and is removed after curing [1]. The resulting polyHIPEs are highly porous (over 75% porosity) with pores that are typically interconnected, making them excellent candidates for tissue engineering [2–4]. Additionally, the morphology and mechanical properties can be modified through adjusting the proportions of a polyHIPE's composition and through changing the synthesis conditions [5,6]. Interconnected porous structures of suitable size enable cellular migration and proliferation, vascularization, and the transport of oxygen, nutrients and metabolic waste [7,8]. In bone tissue engineering, for example, pore sizes are ideally over 50 μm to enable osteoblast ingrowth but to allow for tissue vascularization a pore size of 250 μm is necessary. Although macroporosity has a strong impact on osteogenic outcomes, microporosity also plays an important role as well due to its larger surface area contributing to higher protein adsorption as well as cell attachment and growth [9]. To enable these processes that occur on different length scales we propose the combination of emulsion templating with stereolithography to control the structure of the scaffolds on a hierarchy of length scales. In this case the ~50 μm length scale is controlled by the emulsion process [7,10,11] while the structure on the larger length scale (>100 μm) is controlled by the stereolithography process [12,13].

As scaffolds used in bone tissue engineering applications, bioceramics such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) have a composition and structure very close to that of natural bone mineral. They are therefore considered to be the ideal material due to their significant osteoconductive and osteoinductive properties [14–16]. However, poor mechanical performance limits the use of bioceramics for the regeneration of non-load-bearing bone defects [17]. To overcome these limitations presented by bioceramics, composite polymer based scaffolds containing HA have been explored for the purpose of bone tissue engineering scaffolds. It is well-documented that polymer scaffolds which are modified with HA improve cell adhesion and osteoconductive properties [18–20]. Many methods have been used successfully to make polymer scaffolds incorporating HA [21–24].

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As incorporation of HA is one of the most widely used methods to improve biocompatibility and mechanical performance of scaffolds, development of methods to incorporate HA into polyHIPEs would allow a multifaceted approach to scaffold design, combining the versatility of polyHIPEs and the biocompatibility of HA. Previously, Akayet al. [18] successfully added HA into polyHIPEs by dissolving HA in phosphoric acid, followed by recrystallization in situ using sodium hydroxide. The results indicated that there was a significant increase in osteoblast numbers penetrating into the polymer after modification with HA. However, dissolving HA using acids followed by recrystallisation would affect the crystallinity and structure of the HA which, for bone tissue engineering, is crucial in order to achieve a desirable combination of structural and mechanical properties and biological activity.

The aim of this study was to fabricate composite polyHIPEs containing HA particles (without dissolving HA using acid) and to evaluate the stability of the emulsion and the microstructure and mechanical performance of the resulting polyHIPEs. Scaffolds with multi-scale porosity were prepared from the composite HIPEs using a direct-write UV stereolithography technique developed in our laboratory and reported elsewhere [12].

2. Materials and methods

2.1. Materials

Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, Sigma Aldrich, Dorset, UK) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, Sigma Aldrich, Dorset, UK) were used to fabricate nano-sized HA. Ammonium hydroxide ($NH_3 \cdot H_2O$) was used to adjust the pH value. 2-ethylhexyl acrylate (EHA, Sigma Aldrich, Dorset, UK) and Isobornyl acrylate (IBOA, Sigma Aldrich, Dorset, UK) were used as monomers for the polyHIPEs. Trimethylolpropane Triacrylate (TMPTA, Sigma Aldrich, Dorset, UK) was used as crosslinker. Hypermer B246-SO-(MV) (Croda, UK) was used as surfactant. (2,4,6-trimethylbenzoyl)-phosphine oxide/2-hydroxy-2-methylpropiophenone, 50/50 (Sigma Aldrich, Dorset, UK) was used as the photoinitiator.

2.2. Synthesis

2.2.1. HA synthesis

HA was synthesized using the wet method. The detailed synthesis method is described elsewhere [25]. Briefly, calcium nitrate tetrahydrate and diammonium hydrogen phosphate were separately dissolved in distilled water with the concentration of 0.5 and 0.3 mol/l, respectively. The pH values of the solutions were adjusted to 11 by adding ammonium hydroxide. The ammonium phosphate solution was then dropped slowly into the calcium nitrate solution while stirring. The slurry was stirred for 3 h and then aged for 24 h at ambient conditions. The HA precipitate was obtained and fully washed with deionized water.

2.2.2. PolyHIPE synthesis

PolyHIPEs containing HA were prepared by the photopolymerisation of HIPEs with the compositions shown in Table 1. The oil phase contained 55 wt% EHA and 19 wt% IBOA monomers, 20 wt% TMPTA, 2 wt% surfactant and 4 wt% photoinitiator respectively. The detailed synthesis method is described elsewhere [6]. Briefly, EHA and IBOA were mixed in a glass beaker with TMPTA. The surfactant was then added and left to dissolve in a sonic water bath. Once dissolved, the photoinitiator was added and thoroughly mixed in. These five components constitute the continuous phase of the HIPE.

Table 1
The composition of polyHIPEs containing nano-sized HA at different concentrations.

Sample	Plain	HA-0.2	HA-0.4	HA-0.8	HA-1.6
Total oil phase (ml)	5	5	5	5	5
Aqueous phase (ml)	20	20	20	20	20
HA (g)	0.0	0.2	0.4	0.8	1.6
HA concentrations (%)	0	4	8	16	32
Stable time	>6 h	>6 h	>6 h	>6 h	<0.5 h

HA particles were added into the aqueous phase in order to localise the HA predominantly at the surface of the pores in the resulting polyHIPE. Surface localised bioceramic/polymer composite scaffolds could stimulate cell proliferation and osteogenic differentiation [26]. Different concentrations of HA were used, as shown in Table 1. After mixing, the emulsion was then pipetted into a mould and passed through an automated UV belt curer (GEW Mini Laboratory, GEW engineering UV, Surrey, UK) 4 times to produce monolithic samples of polyHIPE. The samples were then dried in a vacuum oven at room temperature.

Four-layer "woodpile" scaffolds with multi-scale porosity were prepared using a single-photon direct write setup according to a method described fully elsewhere [6]. To write the scaffold, a functionalised coverslip atop a microscope slide was placed onto the stage. The program was then started and additional HIPE added after the completion of each layer. Stereolithography was carried out with a write speed of 1.95 mm/s, spacing of 1 mm and laser current of 2.2 A. Each layer was prepared using 60 μl of emulsion. Once fabrication was completed, scaffolds were soaked in acetone for 15 min to remove uncured HIPE and force out any water.

2.3. Characterisation

Fourier transform infrared spectroscopy (FTIR, Nicolet iS50R) studies were carried out in the range of 400–4000 cm^{-1}. Two scanning electron microscopies (SEM, Philips XL-20 and FEI NOVA450) were conducted to examine the microstructure and investigate how changes in composition affected pore morphology and size. The scaffolds were embedded into paraffin wax, and left them in the fridge with the temperature of −80 °C for use. A microtome (Leica) was then used to section the scaffold with 25 μm thickness, and images were taken with an optical microscope. Thermo Gravimetric Analyzer (TGA, Pyris 1 PerkinElmer) was used to study the weight change during the thermal processes after the samples were Soxhlet-washed with water during 48 h. Specimens were tested for tensile mechanical properties according to a method described elsewhere [6,27] using a BOSE ElectroForce 3200 mechanical testing machine equipped with a 450 N load cell, with an extension rate of 0.02 mm/s, a grip distance of 10 mm, and a maximum extension of 6 mm. Ten replicate samples ($30 \times 6 \times 1.5$ mm) of each composition were tested, the force and displacement measured, and the Young's Modulus (E), Ultimate Tensile Stress (UTS), and Percentage Elongation at Failure (%Elong) determined. Water contact angle testing was evaluated using the sessile drop method. The testing method was carried out according to a method described elsewhere [6].

3. Results and discussion

3.1. Compositions of polyHIPE containing HA

The FT-IR spectrum in Fig. 1 shows the presence of —OH (3569 and 628 cm^{-1}), broad bands of adsorbed water (around 1650 and 3440 cm^{-1}), bands due to PO_4^{3-} ions (559 and 599 cm^{-1}, 961 cm^{-1}, 1020 and 1087 cm^{-1}) in the samples containing HA, which are all bands characteristic of HA [26,28]. The remaining bands correspond well with those of the plain polyHIPE, indicating that HA did not change the chemical structure of the polymer. Above approximately 550 °C no residual weight remained in the plain polyHIPE, but a residual weight was observed in the samples containing HA. The residual fraction must be HA as the polymer would be decomposed completely during this process. Furthermore, the residual weight increased as the