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High internal phase emulsion as reaction medium for precipitating brushite crystals

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Abstract

This present work was aimed at fabrication of brushite crystals using oil-in-water high internal phase emulsion as a reaction medium. The oil phase of more than 75 wt.% was dispersed in the continuous aqueous phase. Due to the high oil volume fraction, the oil droplets were no longer spherical but were squeezed to take the shape of polyhedral. The morphology of the crystals was influenced by the structure of the emulsion and precursor concentration. The crystals were subjected to cytotoxicity test to ensure their compatibility with synoviocytes, which are cells that line the knee joints of rabbits. The crystals were able to sustain the cells for 5 days, which manifest their potential as osteoconductive coatings.

Keywords: E. Biomedical applications; High internal phase emulsion; Brushite

1. Introduction

Chemical analysis shows the presence of calcium and phosphate as principal constituents of natural bone, enamel and dentin. Synthetic calcium phosphates could be fabricated similar to the crystallographic structure of natural bone, enamel and dentin [1]. Calcium phosphates have a wide range of applications in tissue engineering, treatment of bone diseases and controlled drug delivery systems [2]. They have excellent biocompatibility and bone bonding or bone regeneration properties. Moreover, they have the ability to enhance bone growth across a gap around an implant in both stable and unstable mechanical conditions and even convert motion-induced fibrous membrane into a bony anchorage [3].

Porous calcium phosphate ceramic coatings are often applied on strong and load-bearing core materials for biological fixation or osteointegration of load-bearing implants such as hip stems and dental roots. Porous materials are associated with the connective tissue of vertebrae, where they form the main part of the bone. Studies have indicated that pores of calcium phosphates are necessary for controlled bioactivity and bioresorbability [4,5]. Numerous studies have been carried out to investigate calcium phosphate coatings on metal and polymer implants. Calcium phosphates have been used as surface coatings on many types of bioinert metallic and ceramic substrates, such as titanium and alumina [6]. Taniguchi et al. [7] demonstrated that sintered calcium phosphates showed excellent biocompatibility with soft tissues such as skin, muscle and gum, and designed a percutaneous catheter surrounded by calcium phosphates to prevent bacterial infections.

In contact with biological fluids, calcium phosphate ceramics degrade via dissolution–reprecipitation mechanisms [8]. Under physiological conditions, this dissolution process is highly dependent on the nature of the calcium phosphate substrate and their thermodynamic stability, for example (in order of increasing solubility), hydroxyapatite (HA) < tricalcium phosphate (TCP) < octacalcium phosphate (OCP) < bicalcium phosphate dihydrate (DCPD) [9].

Brushite (DCPD) has attracted great interests amongst researchers as a resorbable bone replacement material due to its

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higher solubility at pH 7.4 in physiological conditions compared to other calcium phosphate crystalline phases [10,11]. The biocompatibility and degradation of brushite have been demonstrated in several in vitro and in vivo studies [12–14]. Therefore, brushite is a suitable candidate as a bioceramic coating in addition to the association with the osteoconductivity of calcium phosphates [15]. Moreover, a porous bioceramic external layer on a metallic or ceramic substrate, which is used as a bone implant, will promote bone ingrowth [16]. In treating bone diseases like osteoarthritis, cells could be loaded onto bioceramic-coated matrices before implanting the cell-loaded matrices into a host body for achieving bone tissue regeneration [17].

High internal phase emulsion (HIPE) is gaining considerable interests amongst researchers as a reaction medium for the preparation of meso/macroporous materials [18]. This particular emulsion consists of disperse phase which exceeds the close packing volume limit of 0.74, the point where the droplets just touch each other [19]. Emulsion droplets of sizes between 0.5 μm and 5.0 μm offer a reaction medium fit for the creation of micron-sized pores into an inorganic or organic matrix [2].

In this work, calcium phosphate crystals with unique morphologies were successfully synthesized through palm olein-in-water (O/W) HIPE. Cytotoxicity test was performed on the crystals using synovial intimal cells, termed synoviocytes [20], to investigate their compatibility with the continuous cell line from the periarticular soft tissue lining the knee joints of rabbits. The periarticular soft tissue is subjected to considerable experimental scrutiny owing to its involvement in bone diseases [21–25]. This tissue lining contains a surface layer, known as the synovium, which is 2–3 cells deep. The synovium is underlain by a connective tissue framework with varying amounts of fibrous, areolar and fatty tissues, which is supported by a thick, collagenous tissue called the ''capsule'' [26]. Studies indicate that synovial tissues are enriched with cells of the monocyte/macrophage lineage that, with appropriate stimuli, can be induced to differentiate into preosteoclasts and ultimately into fully functional osteoclasts [27–29]. In addition, several studies have shown that synovial tissues are a rich source of a number of cytokines and inflammatory mediators that possess the capacity to induce the recruitment, differentiation and activation of osteoclasts [30–36]. Designated HIG-82 line was produced by spontaneous establishment of an aging, late-passage culture of primary cells.

2. Experimental

2.1. Calcium phosphate fabrication

In order to fabricate calcium phosphate crystals through the HIPE processing route, two aqueous solutions containing (a) 5.0 wt.% Brij 35 (Fluka) and 0.50 M calcium chloride, $CaCl_2{\cdot}2H_2O$ (Sigma–Aldrich) and (b) 5.0 wt.% Brij 35 and 0.30 M disodium hydrogen phosphate, Na_2HPO_4 (Sigma–Aldrich) were prepared. Brij 35 is polyoxyethylene lauryl ether $(C_2H_4O)_{23}C_{12}H_{25}OH$ with CAS No. 9002-92-0. Refined–bleached–deodorized (RBD) palm olein (Moi Foods Malaysia Sdn. Bhd.) as oil phase was added drop wise into each of the aqueous phase equally while stirring. The oil volume fraction (Ø) was 0.80, which was calculated based on Eq. (1):

$$\varnothing = \frac{m_o/\rho_o}{(m_o/\rho_o) + (m_w/\rho_w)} \quad (1)$$

where m_o/ρ_o is the volume of oil and m_w/ρ_w is the volume of water [37].

The mixtures were then mixed and homogenized at 1500 rpm for 15 min using a Multimix CKL mixer at room temperature to form HIPE, which was allowed to age at 40 °C in an MMM Vacucell vacuum oven for 7 days for the formation of calcium phosphate crystals. The pH of the HIPE reaction medium containing calcium and phosphate ions was determined at the initial and end of the aging period using an Orion Model 420A pH meter fitted with a Ross pH electrode at 25 °C. In order to retrieve the precipitates, ethanol (98%, Fluka) was added to demulsify the HIPE system. The demulsified HIPE was centrifuged using a Hettich centrifuge at 4500 rpm for 30 min to separate the precipitates from the medium. The washing process was repeated three times with ethanol followed by deionized water. The precipitates were then calcined at 600 °C in a Carbolite furnace for 2 h to obtain white powders. The process was repeated with 0.30 M and 0.10 M of $CaCl_2$, and 0.18 M and 0.06 M of Na_2HPO_4, respectively. Bulk calcium phosphate was prepared using conventional wet chemical processing route whereby 0.50 M $CaCl_2$ aqueous solution was titrated with 0.30 M Na_2HPO_4 aqueous solution under constant stirring, as a comparison to the HIPE-prepared calcium phosphates.

2.2. Characterization

Crystallinity of the powders was measured using a Phillips X-ray Diffractometry (XRD). The as-prepared powder was placed on a glass slide. Measurements were taken from 4° to 70° on the 2θ scale at a size step of 0.033° s^{-1}. The XRD data was processed using in-built PANalytical X'pert HighScore software to examine the peak position and its corresponding intensity data. Chemical bonding of the powders was analyzed using a Perkin-Elmer Fourier transform infrared (FTIR) spectroscopy. The powders were mixed with potassium bromate, ground homogenously and converted into pellets. The spectra (% transmittance with wave number) were recorded. The morphology of the powders was observed using a LEO 1455 Variable Pressure Scanning Electron Microscopy (VPSEM). The powders were mounted on aluminum stubs using double-sided tape and vacuum coated with gold in a Polaron SC500 sputter coater.

2.3. Cytotoxicity test

Cytotoxicity test was carried out for the evaluation of cell compatibility in direct contact with brushite crystals. HIG-82 cell line (synoviocytes) was purchased from American Type Culture Collection (ATCC, Manassas, USA) and cultured in the