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In vitro *Evaluation of Mesoporous Carbonated Hydroxyapatite in MC3T3-E1 Osteoblast Cells*

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Abstract

Nanoporous bioceramic recently has gained attention as a drug storage and release host in the therapeutic application. Mesoporous carbonated hydroxyapatite (MCHA) is a nanoporous bioceramic with pore sized ranged between 2-50 nm. In order to use in drug delivery application, this material should demonstrate acceptable cytotoxic activity and osteoinductive response. Therefore, this study aimed to evaluate the effect of mesoporous carbonated hydroxyapatite on viability and alkaline phosphatase (ALP) activity of MC3T3-E1 osteoblasts cell line. These parameters represent cytotoxicity level and osteinductivity capability of the materials. MC3T3-E1 cells were cultured in 25 mg/ml extraction of mesoporous carbonated hydroxyapatite (meso-CHA) and non-porous CHA (np-CHA) for up to 7 days for viability and 14 days for ALP test. Results indicate that meso-CHA shows better cytotoxicity properties compare to np-CHA. Intracellular ALP of cells treated with meso-CHA was higher than np-CHA.

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1. Introduction

Porous materials with ordered structure have become an interesting and popular topic for research in adsorptions since the discovery of Mobil Composition of Matter No. 41 (MCM-41) in 1992[1]. According to International Union of Physical and Applied Chemistry (IUPAC) classification, pores can be categorize as micropore (pore size < 2 nm), mesopore (2 < pore size < 50 nm) and macropore (pore size > 50 nm)[2]. As these pore sizes fall within the nanoscale range, they can also be described as nanopores. Macroporous hydroxyapatite (HA) which have similar chemical composition to human bone and teeth have been utilized as a vehicle for the delivery of pharmaceuticals molecules such as ibuprofen[3-6], alendronate[7], zolendronate[8], vancomycin[9], norfloxacin[10] and carvedilol[11] to the treated area. Macroporous HA exhibited the 'burst' release (the sudden release of a drug in large amounts at the initial stage of the delivery period) profile during drug delivery process[12]. This behaviour is considered as a major drawback for its application in drug delivery. It can be attributed to the fact that drug molecules tend to concentrate on the external surface of dense materials rather than attaching to the internal pores[13]. Consequently, dense and macroporous HA with lower porosity and larger external surface in relative to internal surface area increases the possibility for 'burst' release to occur and thus, less suitable to be used as drug carrier for controlled release. It would be relatively easier for drug molecules to be adsorbed by material with larger surface area and higher adsorption strength. Mesoporous materials have smaller pore size, larger surface area and higher adsorption strength can load more drugs and release in a controllable manner and thus is a much better candidate as a vehicle for drug release.

Carbonated hydroxyapatite is a nonstoichiometric version of HA[14]. The substitution of carbonate in the crystal structure is known to weaken the apatite structure and makes it more soluble[15]. Thus, carbonated hydroxyapatite (CHA), which is chemically more similar to human bone constituents, has therefore shown to demonstrated better biocompatibility, bioactivity, and resorbability[14, 16] compared to HA. For this reason, a better profile of drug adsorption and release is expected from mesoporous CHA. Biocompatibility is the main concerned for biomaterials; therefore all new developed biomaterials must fulfill specific criteria laid out by the government authorities and international agencies, before receiving approval for clinical application. In practice, standard cell-based toxicity assays are performed in-vitro to evaluate cytotoxic level of the biomaterials. It is important to consider the possible impact of the materials on the process linked to cell proliferation and differentiation. Hence it would be of interest to evaluate the cytotoxicity properties of the mesoporous carbonated hydroxyapatite materials and the effect of this material on the cell differentiations. The aim of this study is to determine the level of toxicity of the in-house synthesise mesoporous CHA and to confirm the effect of mesopore within the CHA nanopowders on cell differentiation compared to non-porous CHA.

2. Methodology

2.1 Sample Preparation

Mesoporous carbonated hydroxyapatite (meso-CHA) nanoparticles was synthesise using calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$), and diammonium hydrogen phosphate ($(NH_4)_2.HPO_4$) as calcium and phosphate precursor, respectively. Pluronics® P123 (BASF, USA) was used as non-ionic surfactant to create pore within nanoparticles. As described in our previous paper[17, 18], initially surfactant-calcium ion containing solution was prepared by dissolving 1 g of P123 in 100 mL of deionised water (DI water) followed by the addition of 9.45 g of $Ca(NO_3)_2.4H_2O$. The solution was then stirred for 30 minutes. The source of phosphate ion was prepared by dissolving 3.17 g of $(NH_4)_2.HPO_4$ in 60 ml of DI water. Next, the phosphate ion containing solution was mixed with 3.795 g of ammonium hydrogen carbonate (NH_4HCO_3) as a carbonate ion source. Subsequently, the prior prepared phosphate-carbonate mixture was dripped slowly into the surfactant-calcium containing solution under continuous stirring, producing the milky solution. The alkanity of the milky solution was maintained at pH 11 throughout the mixing process. The milky solutions then poured into a Teflon® bottle and aged at 120°C in an oven for 24 hours. Next, the solution was cooled down and centrifuged at 3000 rpm for 20 minutes to obtain the white precipitate. The excess surfactant was removed from the precipitate by washing and centrifuging for five times with DI water. The white precipitate then dried in an oven at 100°C for 24 hours. The dried precipitate was then ground into fine powders by using mortar and pestle and further calcined in a furnace at 550 °C for 6 hours. In this study, the biocompatibility of synthesise meso-CHA was compared to nonporous carbonated hydroxyapatite (np-CHA). Sample np-CHA was

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