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In vitro study of vancomycin release and osteoblast-like cell growth on structured calcium phosphate-collagen

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ABSTRACT

A drug delivery vehicle consisting of spherical calcium phosphate-collagen particles covered by flower-like (SFCaPCol) blossoms composed of nanorod building blocks and their cellular response is studied. The spherical structure was achieved by a combination of sonication and freeze-drying. The SFCaPCol blossoms have a high surface area of approximately 280 m^2g^{-1}. The blossom-like formation having a high surface area allows a drug loading efficiency of 77.82%. The release profile for one drug, vancomycin (VCM), shows long term sustained release in simulated body fluid (SBF), in a phosphate buffer saline (PBS, pH 7.4) solution and in culture media over 2 weeks with a cumulative release ~53%, 75% and 50%, respectively, over the first 7 days. The biocompatibility of the VCM-loaded SFCaPCol scaffold was determined by *in vitro* cell adhesion and proliferation tests of rat osteoblast-like UMR-106 cells. MTT tests indicated that UMR-106 cells were viable after exposure to the VCM loaded SFCaPCol, meaning that the scaffold (the flower-like blossoms) did not impair the cell's viability. The density of cells on the substrate was seen to increase with increasing cultured time.

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1. Introduction

Open fracture of bone due to traumas and the use of implants to repair bone damage arising from bone fracturing or from diseases have created the need to develop biomaterials which can deliver drugs or bone growth factors to specific sites [1–3]. One would need a material which would be biocompatible with the human body and be similar to the mineral component of bone tissue in order to achieve direct chemical bonds between different bone parts and between the bone and the implanted material. Calcium phosphate (CaP) especially hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ (HAp) is the material of first choice since it is the main component of natural bone and as such has the required biocompatibility and osteoconductivity [4–6]. In addition, CaP has been used to make vehicles for drug delivery [7,8] and gene delivery [9,10].

The disadvantage of using CaP is the shortness of the release period when used to deliver drugs. Composites of calcium phosphate and polymers have overcome some of this disadvantage. Vehicles made with these composites and having various biomolecules attached to them has been shown to yield sustained releases of biomolecules such as antibiotic drugs [11,12]. In hopes of achieving a drug delivery system (DDS), investigations of composites containing porous scaffolds and core-shell structures are underway to see whether they can be used as a DDS for controlled release [13–16]. The drug loading capacities (DLCs) and lifetime for sustained release need to be determined. To achieve further improvements, studies have looked at hierarchically structured particles as materials which can be used for drug delivery [17–19]. Hierarchically structured particles are obtained when nanosize rectangular blocks, nanosize plate-like blocks or nano rod-like blocks are assembled together. The packing of these types of blocks results in the final particle having high specific area, mesopores and interconnected macro pores. Particles with these three features would be excellent for loading drugs on [17–19]. Since it is a major organic component in natural bone, collagen (Col) has been used as an excellent basis biocomposite scaffold for cell growth and delivery systems [20]. Nudelman et al. [21] recently found that the biomineralization of hydroxyapatite in bone was greatly enhanced by the presence of a collagen scaffold. Meanwhile, Cölfen et al. [22] pointed out that the collagen scaffold would act as a template for attaching the apatite building blocks. The formation of a collagen scaffold within a nano volume would provide a scaffold to attach the different types of nano blocks (rectangular blocks, plate-like blocks and rod-like blocks) to form the hierarchically structured particles. Although previous studies successfully synthesized calcium phosphate-collagen composite but only Anton et al. [23] has looked at the influence of the ultra

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sonication on the synthesis calcium phosphate of hydroxyapatite/collagen composite materials. In light of their work and the fact that calcium phosphate-collagen particles can be used as a delivery system and for cell support, we have sought to obtain better scaffolds for attaching drugs to by first generating the bubble collagen by subjected them to high intensity ultra sonication. We then added drop by drop a calcium phosphate solution to the collagen solution. The resulting mixture was then freeze-dried. This gave spherical-like calcium phosphate-collagen particles on which flower-like (SFCaPCol) blossoms consisting of nanorod building blocks were attached. The resulting structure (morphology) is different from that of Anton [23] (theirs is an agglomeration of spherical like particles). The blossoms service as the scaffold to which drugs molecules could be attached. In the present study, the drug used was vancomycin (VCM). VCM is a broad-spectrum glycopeptide antibiotic which is active against Gram-positive organisms [24–28]. The release kinetics of VCM from a drug loaded SFCaPCol composite in simulated body fluid (SBF), in a phosphate buffer saline (PBS, pH 7.4) solution and in culture media was investigated for period of two weeks. The VCM drug-loaded SFCaPCol composite was then used as the substrate for the growth of rat osteoblast-like UMR-106 cells. The biocompatibility of VCM drug-loaded SFCaPCol composite has been examined by observing the interactions between these cells and the synthesized material surface.

2. Materials and method

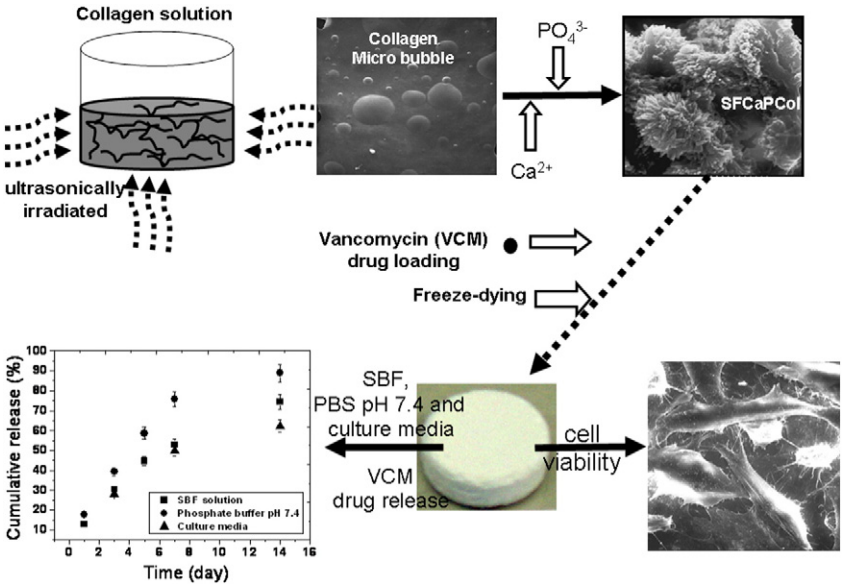
Collagen used in this experiment was made from calf skin (Sigma, USA). $CaCl_2$ (Fluka) and K_2HPO_4 (Fisher Scientific, UK) were used as the precursors of calcium and phosphate, respectively. All chemicals were of analytical grade and used without further purification. The water used throughout this work was de-ionized water. For the experiments on the drug loading and drug release, the drug vancomycin (VCM) (Fluka, Biochemika) was used as a model antibiotic drug.

2.1. Composite preparation

The preparation of spherical-like calcium phosphate-collagen having flower-like blossoms to form nanorod assembly (SFCaPCol) composite particles is shown in Scheme 1. In the formation of the SFCaPCol composites, the collagen acts as an organic template for the nucleation of the CaP crystals. The composite of CaP on the collagen matrix was initiated via a co-precipitation process from solution under ultrasonication. For our preparation, 3 mg/mL of collagen was dissolved in dilute acid (1 mM HCl) and stirred until it was completely dissolved (stroke solution). Ultrasonication is applied according to previous report [17,23]. Briefly, 25 mL of collagen solution was neutralized by phosphate buffer saline (PBS) (pH 7.4) and then ultrasonication (20 kHz [17], Sonopuls HD2200) in an ice bath with temperature about 8 °C during the reaction process (Collagen bubbles are formed in this process). After 5 min, 10 mL of 0.4 M $CaCl_2$ aqueous solution was added to the collagen solution. The mixture was allowed to stand at temperature of 8 °C. The solution at this stage is called Col – Ca. Next, 10 ml of 0.24 M K_2HPO_4 aqueous solution is slowly dropped into the Col – Ca solution (the ratio of Ca/P being 1.67). The mixture was continuous ultrasonication for 5 min. At this point, a white precipitate formed. Following an incubation period, the SFCaPCol composite was then freeze-dried.

2.2. Characterizations

The crystal structure of the composite powders was determined by a powder X-ray diffraction (XRD) (Bruker diffractometer, Model D8 Advance) using the $CuK_{\alpha 1}$ radiation operating at 40 kV and 40 mA. Powder XRD patterns were scanned over the range of $2\theta = 20°$ to 60° at a scanning speed of 1 step per second with step increments of 0.037°. The crystal size in the direction perpendicular to the crystallographic plane of SFCaPCol determined based on Scherer's formula, $d = k\lambda/\beta \cos\theta$ [36], where d is the average crystallite size, λ is the wavelength of X-ray radiation (0.154056 nm), k is a constant related to the crystal shape and is approximately equal to unity. β is the full width of the peak at half of the maximum intensity (rad) and θ is the Bragg' angle. The IR spectra were measured with a Fourier transform infra-red (FT-IR) spectrophotometer (Spectrum GX, Perkin Elmer) which performed 16 scans over the range of 370–2200 cm^{-1}. A scanning electron microscope (SEM) (JEOL model JSM-6301 F) is used to observe the morphology of the composite. An accelerating voltage of 15 kV is used to obtain the SEM images. Nitrogen adsorption experiment was carried out at 77 K using Autosorb-cl analyzer (Quantachrome Instruments). The specific surface areas of SFCaPCol



Scheme 1. Flow chart for the synthesis of a spherical like calcium phosphate-collagen with flower-like (SFCaPCol) composite and vancomycin (VCM) drug loading for controlled release and cell bioactivity.

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