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Hydroxyapatite mineral tubes developed for the loading and release of biological proteins

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ABSTRACT

Hydroxyapatite (HA) mineral tubes developed through the replication of polymer nanofiber mesh were studied for loading and delivery of biological proteins. With varying heat-treatment temperature (500, 800 and 1100 °C), the physicochemical properties of the HA mineral tubes differed substantially, i.e., while crystallinity and crystallite size increased the surface area decreased with increase in the temperature. All mineral tubes retained negative surface charges (around −10 mV) at physiological conditions. Cytochrome C (cyt C), used as a model protein of being positively-charged, was adsorbed well onto the HA mineral tubes, with adsorption being saturated within a few hours. The adsorption amount was significantly higher in the case treated at lower temperature. The cyt C showed a similar release pattern from the HA mineral tubes, with an initial rapid release for a few hours followed by a reduced rate over ~24 h. Results indicated the HA mineral tubes might be tailored to properly load and delivery biological molecules for bone repair.

1. Introduction

Nanomaterials including nanoparticles, nanotubes, and nanofibers have gained great interests in biomedical applications, and particularly as the reinforcing agents and drug delivery vehicles for therapy and diagnostics [1–4]. When the nanomaterials have the capacity to load and deliver therapeutic molecules, the potential to find usefulness in the repair and regeneration of damaged and diseased tissues will be significantly improved [5]. Among the choices of nanomaterials, hydroxyapatite (HA) mineral nanoparticles have been the most fascinating material in biomedicine, particularly for the repair of hard tissues including bone and tooth [6–8]. The excellent biocompatibility, including *in vitro* cell viability and osteogenic stimulatory roles of progenitor and stem cells as well as *in vivo* tissue compatibility and bone forming ability, has enabled extensive uses of HA as bone repair grafts and tissue engineering scaffolds [9–11]. Different forms have specifically been developed either as scaffolds for cultivating cells for tissue engineering, as additives to polymeric matrices, or as delivering vectors of drugs and proteins [12,13].

In the latter case, the capacity to load drug/protein molecules as well as the release pattern over time is essentially considered to achieve appropriate therapeutic effects. Previously, the authors developed a tubular form of HA crystals by the replication technique of a polymeric nanofibrous structure through mineralization and thermal treatment [14]. We consider that when the HA tubes have the ability to deliver therapeutic molecules, particularly proteins, their applications will be greatly improved. We first develop the HA tubes with different crystalline properties, by altering the thermal treatment temperature. The adsorption and release patterns of protein molecules on the different HA mineral tubes are addressed, which will ultimately provide useful information for future therapeutic applications of the HA tubes in bone regeneration.

2. Experimental part

As the template for mineralized tubes, polycaprolactone (PCL) electrospun nanofiber mat was first produced, as described in our previous work [15]. In brief, 10% w/v PCL (M_w=80,000, Sigma) dissolved in dichloromethane/ethanol (4:1) was electrospun at a field strength of 10 kV/10 cm and an injection speed of 0.5 ml/h. For the apatite mineralization, the nanofiber mat was dipped in 2 N NaOH solution while stirring gently for 6 h, alternatively soaked in 150 mM $CaCl_2$ and 150 mM Na_2HPO_4 solution, and then immersed in a simulated body fluid (SBF) for 7 days. The

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mineralized PCL mat was then heat-treated at varying temperatures; 500, 800 and 1100 °C. The morphology of the mineral tubes was observed by field-emission scanning electron microscopy (TESCAN, MIRA II LMH, Czech Republic). The crystalline phase of samples was analyzed by X-ray diffraction (XRD) using an Ultima IV apparatus (Rigaku, Japan) using with CuKα radiation ($\lambda = 1.5418$ Å). X-ray was generated at 40 mA and 40 kV, and data were obtained at diffraction angles (2θ) from 4–70° with a step size of 0.02° and a scanning speed of 2°/min. Based on the XRD diffraction patterns, the crystallinity was defined using the equation as follows [16]: $X_c = (1 - V_{112/300})/I_{300}$, where I_{300} is the intensity of (300) peak and $V_{112/300}$ is the intensity of the hollow between (112) and (300) peaks. Furthermore, the crystallite size was calculated according to Scherrer's equation: $D = k\lambda/\beta \cos\theta$ [17], where λ is 1.5418 Å; k is the shape factor and assigned a value of 0.89 if shape is unknown; θ is the Bragg angle; and β is the full width at half maximum in radian. The most intense peak ($2\theta = 32°$) was selected to calculate the crystallite size. Surface electrical potential was examined by the zeta (ζ) potential measurement using a Zetasizer Nano ZS laser Doppler electrophoresis (LDE) instrument (Malvern Instruments, UK). Samples were dispersed in deionized water at pH 7.0 with a gentle agitation, and the ζ-potential was measured at 25 °C with applied field strength of 20 V/cm. Specific surface area was determined based on N_2 adsorption–desorption measurements. The N_2 adsorption–desorption isotherms were obtained at 77 K using a Quadrasorb SI automated surface area analyzer (Quantachrom Instruments, UK). Samples were degassed under vacuum at 300 °C for 12 h prior to analysis. The specific surface area was determined according to the Brunauer–Emmett–Teller (BET) method [18].

For the loading of biological proteins, cytochrome C (cyt C) was used as the model protein. This was based on our preliminary tests of using either cyt C or bovine serum albumin (BSA), where only cyt C was observed to be effectively loaded. Prepared HA mineral tube samples were soaked in 1 ml PBS, which containing 0.2 mg of cyt C, and incubated in a 37 °C water bath. After adsorption for different time points (1, 3, 6 and 12 h), the supernatant was gathered and then the cyt C quantity was measured by UV detection, according to the manufacturer's instructions. The amount of cyt C adsorbed to the nanotube samples was calculated based on the standard curve obtained. Cyt C release from the samples was performed in PBS. After incubation for different time points (1,

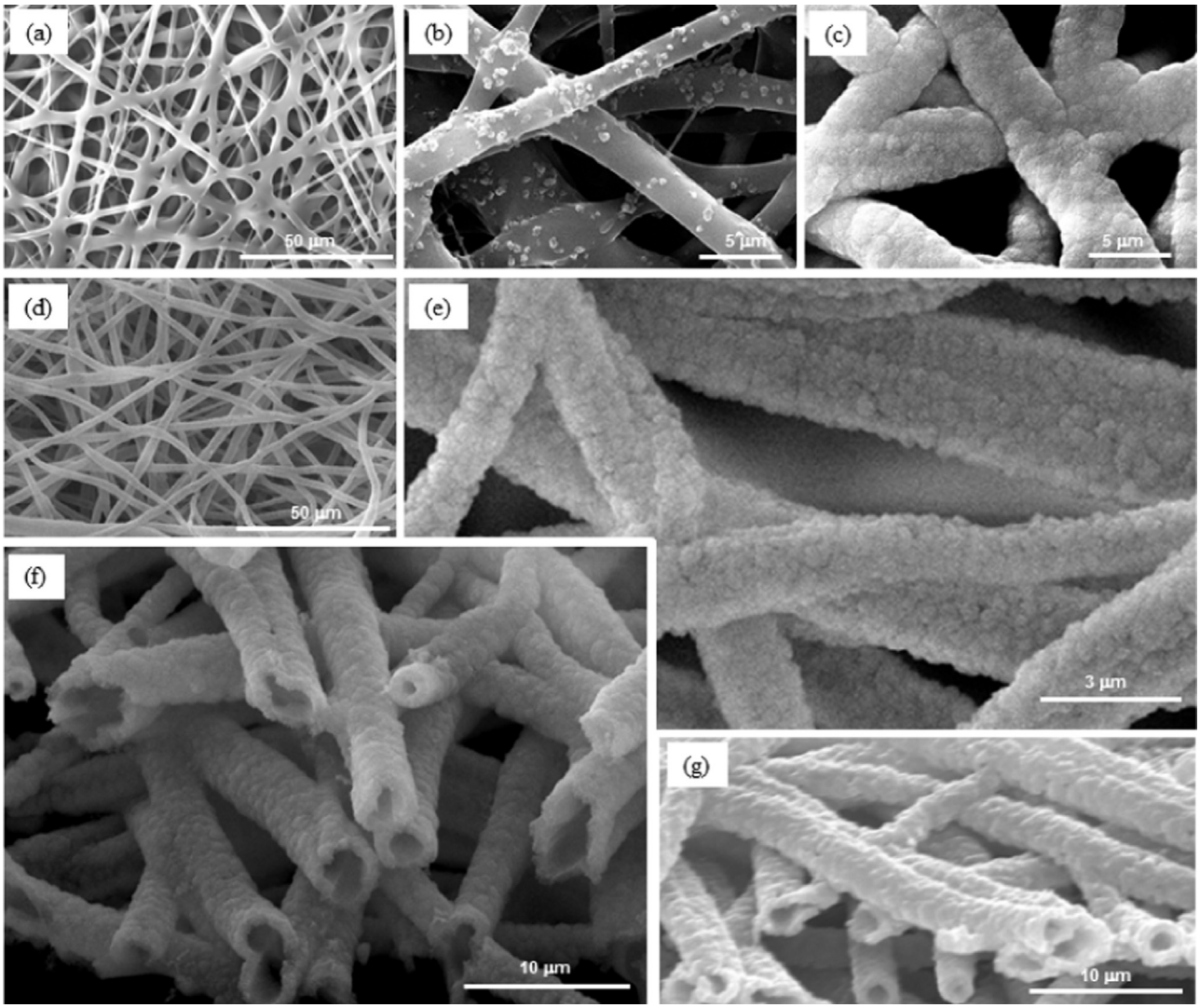

Fig. 1. SEM morphologies of the samples from the starting fiber mesh (a) to surface mineralized fiber (b, c) and to hollow mineral tubes (d–g). (a) Initial PCL nonwoven fibrous mesh obtained by electrospinning. (b, c) fiber surface was mineralized for (b) 1 day and (c) 7 days, and (d–f) subsequently heat-treated at 500 °C at low (d) and high magnification (e), 800 °C (f), and 1100 °C (g). While the mineralization for 1 day showed initiation of crystal formation on the fiber surface (b), prolonged period of 7 days completely covered the surface with nanocrystalline mineral phase (c). Heat-treatment process well preserved the fibrous structure with complete removal of the inner polymer part as revealed by the cross-sectioned images (f, g).