Preparation of strontium- and/or zinc-doped hydroxyapatite nanoparticles and their polycaprolactone composite fibrous scaffolds

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Abstract. In this work, hydroxyapatite (HA) and hydroxyapatite doped with Sr (HA-Sr), Zn (HA-Zn) and both Sr-Zn (HA-SrZn) were synthesized by a sol-gel method and combined with polycaprolactone (PCL) to make HA/PCL composites using an electrospinning technique. The synthesized nanoparticles and their composite fibers were investigated using various techniques. The X-Ray Diffraction (XRD) result showed the characteristic peaks of the hydroxyapatite structure; whereas the scanning electron microscopy (SEM) and transmission electron microscopy (TEM) results revealed that the synthesized nanoparticles were successfully incorporated into the randomly interconnected and highly porous PCL matrix.

1. Introduction
An ideal scaffold for bone regeneration should mimic both the structure and mechanical properties of the natural bone. It should also be bioresorbable with a controllable degradation rate to match that of the regeneration rate of the newly-formed bone tissue [1]. Hydroxyapatite (HA) has received high attention due to its chemical similarity to the inorganic matrix of natural bone, excellent osteoconductivity and bioactivity. However, pure HA have low bioactive property due to their low resorbability [2]. Many studies on modifying HA composition have been conducted to improve its properties such as bioactivity, degradation behavior and antibacterial property [3-6].

In this work, strontium (Sr) and zinc (Zn) were chosen to be doped into the structure of HA due to their noble characteristics. Sr plays an important role in the treatment of osteoporosis and enhancement of bone remineralization as it is associated with a reduction of bone resorption and an increase of new bone formation [5] while Zn plays a vital role in the biochemistry of bone tissues and exhibits antibacterial activity [6]. Poly(ε-caprolactone) (PCL) was chosen to be a matrix for composite fiber since it is a biocompatible and has been widely used in many biomedical applications [7].

2. Materials and Methods

2.1. Materials
Calcium nitrate tetrahydrate (CNT, 99.0% purity), Diammonium hydrogen phosphate (DAHP, 99.0% purity) were purchased from Qrec, Malaysia. Strontium acetate (SA, 99.9% purity), Zinc acetate (ZA, 99.9% purity) and Poly(ε-caprolactone) (PCL, MW=80,000) were obtained from Sigma-Aldrich, USA. Dichloromethane (DCM, 99.9% purity) and N,N-dimethylformamide (DMF, 99.8% purity) were purchased from Showa, Japan. Ethanol (95.0% purity) was supplied by Merck.

2.2 Preparation and general characterization of HA, HA-Sr, HA-Zn and HA-SrZn particles

HA, HA-Sr, HA-Zn, and HA-SrZn powders were synthesized using a sol-gel method [8]. The precursors were mixed at the (Ca+Sr/Zn):P mole ratio of 10:6 and at the Ca: Sr/Zn ratio of 9:1. In the case of HA-ZnSr, the Sr:Zn mole ratio was 1:1. CNT was dissolved in ethanol, and where applicable SA and/or ZA were added. DAHP was separately dissolved in deionized water. The two solutions were quickly mixed thoroughly after which the gel was formed. The gel was then aged for 24 hours at room temperature and heated at 80 °C. The obtained powder was then calcined at 600 °C for 2 hours.

The X-ray diffractometer (XRD, D8 ADVANCE, Bruker) was used to identify the phase structure of the calcined powders. The XRD patterns were obtained in a 2θ angle ranging from 20° to 80° at a scanning speed of 0.02°/min. To observe their morphologies, the calcined powders were dispersed in ethanol by sonication for 15 min, dropped onto the carbon coated copper grids and air-dried for a transmission electron microscopy (TEM, TECNAI G220, FEI) observation.

2.3 Electrospinning and general characterization of PCL/doped and undoped-hydroxyapatite composite fibrous scaffolds

A 10% w/v PCL solution was prepared by dissolving PCL in a DCM/DMF mixture (DCM:DMF volume ratio of 20:80). The HA, HA-Sr, HA-Zn or HA-SrZn powder was then added into the PCL solution at the HA:PCL weight ratio of 1:10 and mixed well for 3 hours using an ultrasonic bath.

The PCL/HA composite fibrous scaffold was prepared by electrospinning (KKU ElectroSys) of the mixture suspension. The as-prepared suspension was added into a 10 ml plastic syringe mounted on a syringe pump in which the needle was connected to a high-voltage supply. Under 18 kV voltage, the fluid jet was injected out at a rate of 1.0 ml/h and the resultant composite fibers were collected on an aluminum foil at the distance of 20 cm from the needle. The morphologies of the fibrous scaffolds were studied using a scanning electron microscope (SEM, S-3000N, Hitachi) and a TEM. For the TEM observation, each scaffold was sputter-coated with a thin layer of gold. For the TEM observation, the fibers were electrospun directly onto the carbon coated copper grids and air-dried.

3. Results and Discussion

3.1 Phase and morphology of the doped and undoped hydroxyapatite particles

Figure 1 shows the XRD patterns of the synthesized powders. Hydroxyapatite (JCPDs 74-0566) was found to be the main phase for all compositions with β-tricalcium phosphate (JCPDs 09-0169) as a second phase. In addition, α-tricalcium phosphate (JCPDs 09-0348) and CaZn₂(PO₄)₂(H₂O)₂ (JCPDs 86-2372) were also observed in the HA-Zn and HA-SrZn powders.

The morphology of synthesized particles can be seen from the TEM images in Figure 2. In general, the undoped HA particles are relatively equiaxed. The particle sizes are approximately in the range of 80-280 nm, with the average of 151±47 nm. More variety can be observed in cases of doped HA samples both in terms of particle shape and size. In addition to the equiaxed grains, rod-like particles are also evident, most prominently in the case of HA-SrZn. The size of the equiaxed particles appear to be smaller than those of the pure HA, as observed to be in the range of 50-200 nm and with the averages of 116±31, 86±45, and 94±31 nm in diameter for the HA-Sr, HA-Zn and HA-SrZn samples, respectively. The width and the length of the rod-like particles are approximately 50-200 nm and 150-300 nm, respectively, and an aspect ratio is approximately 1.8. This is consistent with the work of Hu et al. where rod-like Zn-doped HA nanoparticles were observed [9]. Similar result was also found in
the work of Qaisar et al. where rod-like Sr-doped HA particles were found [3]. This elongation associated with Sr doping is due to the substitution of Ca ions (180 pm) with Sr ions (200 pm).

![XRD pattern of HA, HA-Sr, HA-Zn and HA-SrZn powders.](image)

**Figure 1.** XRD pattern of HA, HA-Sr, HA-Zn and HA-SrZn powders.

### 3.2 Morphology and inner structure of electrospun scaffolds

The SEM and TEM images of the electrospun fibrous scaffolds are shown in figure 3, respectively. From the SEM images, it can be seen that all the electrospun scaffolds exhibited a randomly interconnected and highly porous structure which resemble the extracellular matrix (ECM) of bone. The average diameter of the pure PCL fiber is approximately 612±3 nm. With an addition of HA particles, the average fiber diameters increase to 675±3, 674±2, 669±3 and 687±1 nm, for the PCL/HA, PCL/HA-Sr, PCL/HA-Zn, and PCL/HA-SrZn composite fibers, respectively. All these sizes fall in the range of collagen fibers of bone which is 100–2000 nm [1] and enables the scaffolds with a structural similarity.

The surface of pure PCL fiber is relatively smooth, whereas those of the composite fibers are rough with particle protuberances. The TEM images reveals the inner structure of the composite fibers with the presence of the HA particles internalized or on the sub-surface of PCL fibers. This confirmed that the HA, HA-Sr, HA-Zn and HA-SrZn particles were successfully incorporated into the PCL matrix. However, agglomeration of the particles can be observed. This is likely because HA particles have low surface charge [8] resulting in a low electrostatic repulsive force between them.

### 4. Conclusions

In this study, Sr and Zn was doped or co-doped into the structure of hydroxyapatite by a sol-gel technique and characterized by the XRD and TEM. An electrospinning process was used to prepare the pristine PCL fiber and PCL/HA, PCL/HA-Sr, PCL/HA-Zn and PCL/HA-SrZn composite fibers. The composite fibers of PCL with doped HA may be expected to help enhancing the osteoblast growth in bone tissue regeneration.

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Figure 2. TEM images of HA, HA-Sr, HA-Zn and HA-SrZn powders.

Figure 3. (a) SEM images and (b) TEM images of PCL fiber and composite fibers.

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