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Synthesis of calcium-phosphate microsphere by ultrasonic spray-pyrolysis technique and its application to drug delivery system

超音波噴霧熱分解法によるリン酸カルシウム微小球の合成と薬剤送達システムへの応用

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1. Introduction

Synthetic clcium phosphate based materials such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HAp) and tricalcium phosphate ($Ca_3(PO_4)_2$; TCP) are compounds with high potential for clinical applications to orthopedic and dental region. We have synthesized the HAp and the apatite-family compounds by an ultrasonic spray-pyrolysis technique.

An ultrasonic spray-pyrolysis technique is one of the fine particle preparation processes via liquid phases. This technique has an advantage that one can prepare stoichiometoric and homogenous compounds by spraying the solutions with desired concentrations of metallic ions into the hot zone of an electric furnance. In addition the above process has also the following advantages: (i) simple process, (ii) narrow particle distribution of the resultant particles and (iii) the formation of spherical and hollow particles.

We have applied the resulting hollow microspheres as a carrier of a drug delivery system (DDS) for medical treatment of cancers using an anti-angiogenic agent, TNP-470, as one of drug models[1]. In the present study, we examined a process to form nano-pores on the microspheres surface for synthesis of the microspheres which could release a drug efficiently.

2. Materials and Methods

Preparation of calcium-phosphate microspheres.

As previously reported[2], the apparatus for the ultrasonic spray-pyrolysis was comprised of an atomizer, a heating part (mullite tube: 2.5 cm in I.D. and 1 m in height; electric furnace: 3 cm in I.D. and 60 cm in height), a powder collecting part (test-tube type filter) and a controller. In addition, the heating part in this apparatus was comprised of two electric furnaces; the lower furnace for evaporation of the solvent from the droplets and the upper one for the pyrolysis. An aspirator was used to introduce the droplet into a heating part at a suction rate of 1.5 $dm^3 \cdot min^{-1}$.

The starting solutions with a Ca/P ratio of 1.50 was prepared by mixing $Ca(NO_3)_2 \cdot 4H_2O$, $(NH_4)_2HPO_4$ and HNO_3 so that their concentrations were 0.60 $mol \cdot dm^{-3}$, 0.40 $mol \cdot dm^{-3}$ and 0.10 $mol \cdot dm^{-3}$, respectively. The concentrations of NaCl in the starting solutions were in the range of 0.50 to 1.00 $mol \cdot dm^{-3}$. The upper and lower furnace temperatures were fixed at 850 and 300 °C, respectively. These solutions were sprayed into the heating zone using the ultrasonic vibrator with the frequency of 2.4 MHz and then the sprayed droplets were dried and pyrolyzed to form the calcium-phosphate microspheres including NaCl phase. The resulting microspheres were washed with pure water, and freeze-dried to prepare the "washed powder".

As-prepared and washed powders were characterized by X-ray diffractmetry (XRD), Fourier transform infrared spectrometry (FT-IR) and scanning electron microscopy (SEM).

Release behavior of TNP-470 from calcium-phosphate microsphere.

TNP-470 was dissolved in ethanol to concentrations of 2000 μg/ml. TNP-470 was loaded by adding the microspheres (0.06 g) into the above mentioned ethanol solution (12 cm^3) and freeze-drying followed. The release behavior of the TNP-470 agent from the resulting TNP-470 loaded microspheres was examined as follows. The TNP-470 loaded microspheres (25 mg) were immersed into physiological saline (0.5 cm^3) at 37 °C for 0.5, 1, 3, 5, 7, 9, 12, 24 and 48 h during shaking at rate of 100 stroke/min. After the desired immersion periods, the suspension was centrifuged at 1000 rpm for 5 min. The amount of TNP-470 in the supernatant was determined by high performance liquid chromatography (HPLC).

3. Results and Discussion

Figure 1 shows the XRD patterns of the resulting powders and the washed powders in the series of 1.00 $mol \cdot dm^{-3}$ in the NaCl concentration as representative examples. The XRD patterns

indicated that the crystalline phases of as-prepared powders were apatite/NaCl biphase with low crystallinity and washed powders were apatite phase with low crystallinity.

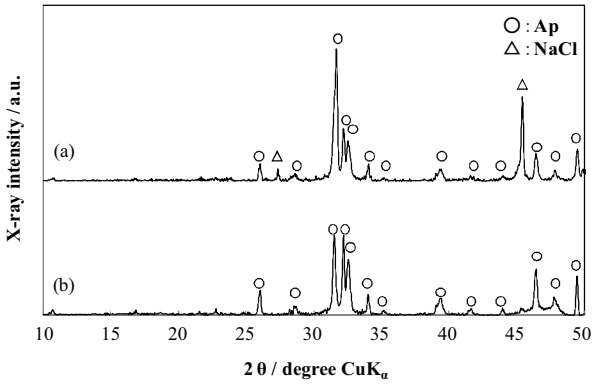

Fig. 1 The XRD patterns of the resulting powders (a) and the washed powders (b) in NaCl concentration 1.00 mol·dm^{-3}.

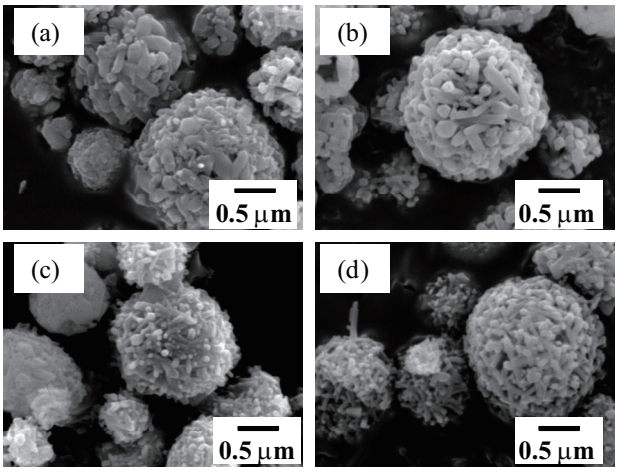

Fig. 2 SEM images of resulting powders (a), washed poweders (b) in NaCl concentration 0.60 mol·dm^{-3}, and resulting powders (c), washed powders (d) in NaCl concentration 1.00 mol·dm^{-3}.

Figure 2 shows typical particle morphologies of as-prepared and washed powders. About 80 % of the whole was consisted of spherical particles, and a rod-shaped particle was present on the microspheres surface. In addition, the SEM micrographs indicated that as-prepared and washed powders were composed of microspheres with a diameter of 0.5-5.0 μm, and the fine pores with the sizes of ~50 nm were present in the surface of the microspheres.

Figure 3 shows the drug release profile of the TNP-470 loaded microspheres. In NaCl concentration of 1.00 mol·dm^{-3}, 70 % of the total TNP-470 from the microspheres was rapidly released within 1 h, and the remaining 30 % was slowly released up to 24 h following immersion, and the total amount of TNP-470 released from the microspheres was 31 μg·mg^{-1}. In addition, the microspheres with nano-pores in the surface showed two steps drug release behavior.

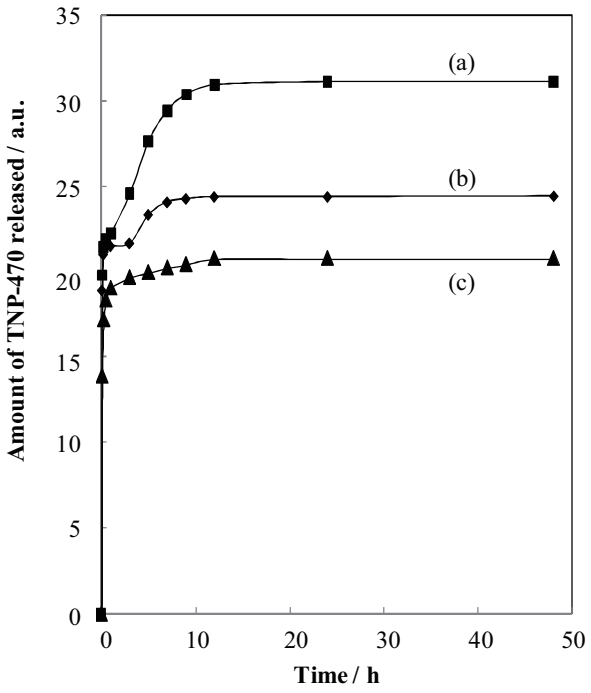

Fig. 3 The drug release profile of the TNP-470 loaded microspheres, NaCl concentration of 1.00 mol·dm^{-3} (a), 0.60 mol·dm^{-3} (b), and no NaCl addition. (c)

4. Conclusion

Hollow and porous calcium-phosphate microspheres were prepared by salt-assisted ultrasonic spray-pyrolysis technique, and aimed to form nano-pores on the microspheres surface by changing NaCl concentration of starting solution, and characterization of as-prepared and washed powders was carried out. The both powder of diameter range from 0.5 to 5.0 μm, and the nano-pores with the size of ~50 nm were present in the surface of the microspheres. The drug release profile of the TNP-470 loaded microspheres showed that the microspheres with nano-pores in the surface showed two steps drug release behavior. We can conclude that calcium-phosphate microspheres prepared by salt-assisted ultrasonic spray-pyrolysis technique are effective for a DDS carrier.

References

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