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Fabrication and characterization of novel porous titanium microspheres for biomedical applications

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ABSTRACT

Microsphere systems have been widely used in the treatment of defective tissues, including bone, cartilage, and muscle. Although researchers are interested in porous calcium–phosphate microspheres, no articles that discuss the preparation of porous Ti microspheres are present in the literature. In the current study, porous Ti microspheres were successfully fabricated via a water-in-oil emulsion technique using camphene as a porogen and a tailored sintering schedule. The results show that the porous Ti microspheres exhibited an interconnected porous structure with pore sizes ranging from several microns to as large as 200 μm, and with various porosities from 49.1% to 74.3%. More than 65% of the pores were larger than 50 μm. Additionally, the surfaces of Ti microspheres were modified with NaOH treatment, and then the calcium–phosphate forming ability in simulated body fluid (SBF) solution was investigated to assess bioactivity. After NaOH treatment, the Ti microsphere exhibited a porous network structure, which is a sodium hydrogen titanate reaction layer as confirmed by the XRD results. A dense and uniform calcium–phosphate layer covered the surface of the NaOH-treated Ti microsphere after it was soaked in SBF solution for 14 days. However, there is no obvious formation of calcium–phosphate on the surface of the untreated Ti microsphere even after it was soaked in SBF for 14 days.

1. Introduction

There is increasing interest in fabricating porous scaffolds that mimic the architecture of bone; osteoblasts obtained from a patient's hard tissue can be expanded in culture and seeded onto scaffolds where they gradually integrate with new bone tissue in vitro or in vivo [1]. Porous calcium–phosphate ceramics can be osteoconductive as well as osteoinductive when their porous structure is controlled appropriately [2]. However, the materials have poor mechanical properties: they are usually very brittle and prone to fracture upon sudden impact, particularly during the healing stage [3]. It is therefore important to develop scaffold implant materials with both reliable mechanical properties and porous structures that are similar or superior to natural bone.

Metallic materials have been widely used in orthopedics for many years, especially for high load-bearing applications, such as dental, maxillofacial, spinal, femur, and knee joints, and bone plates and screws. Ti and its alloys are of particular interest to researchers because of their relatively low density, high strength-to-elastic modulus ratio, outstanding corrosion resistance, and better biocompatibility [4–6]. Additionally, as Wen et al. [7] have revealed, Ti and Ti alloy foams are not only biocompatible, but the material's open-cellular structure also permits the ingrowth of new bone tissue and the transport of body fluids. The mechanical properties of cellular structures can be adjusted by modifying the amount, morphology, and size of the pores. As expected, a more pronounced response was observed in the porous rather than the solid materials. Hsu et al. [8] reported that the inner surfaces of NaOH-treated porous Ti–7.5Mo specimens have a better apatite-inducing ability, and their cell culturing results revealed that the osteoblast-like cell MG63 had extended pseudopodia and was attached well inside the pores after 7 days of cell culturing.

More recently, calcium–phosphate microspheres have received a lot of attention as controlled drug delivery systems [9]. From the macroscopic viewpoint, macroporous microspheres can better perform their role as a cell carrier. As in the case of porous scaffolds, they can facilitate vascularization and nutrient supply, and can populate and deliver as many cells as possible [10,11]. Unlike block-type porous scaffolds, the microspherical form can be applied as an injectable system for minimally invasive surgery [12]. Du et al. [13] fabricated porous TiO_2 microspheres with specific surface areas by a facile hydrothermal method. Kim et al. [14] reported a golf ball-shaped PLGA microparticle with internal pores fabricated by simple oil-in-water emulsion. Hong et al.

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[12] prepared polycaprolactone microspheres containing interconnected pore channels, with a porous surface tailored with bone-bioactive apatite, for application in the augmentation and tissue engineering of bone. They demonstrated that cells were populated better on the porous microspheres than on the dense microspheres after all culturing periods. Porous ceramic and polymer microspheres have been widely reported over the past few years; however, very few studies are found in literature concerning porous Ti microspheres at present. Walschot et al. [15] assessed the feasibility of using porous Ti particles for impaction grafting. Their porous Ti particles with heterogeneous shapes ranging from round to irregular were produced during the purification of Ti through titanium tetrachloride. Moreover, they indicated that osteoconduction in the bone conduction chamber was reduced more by the insertion of small Ti particles than by insertion of small allograft bone particles [16].

In the present study, porous Ti microspheres were successfully fabricated via a water-in-oil emulsion technique using camphene as a porogen and were synthesized using a tailored sintering schedule. Camphene is non-toxic and is commonly used in the preparation of fragrances. Recently, the material has also been studied to examine its potential for use in the processing of ceramic scaffolds such as alumina and hydroxyapatite [17,18]. Importantly, camphene easily sublimes under ambient conditions because it solidifies at approximately 40 °C and has a high vapor pressure [17]. This ensures effortless pore generation. In the current study, pure Ti powders were combined with a binder of polyvinyl alcohol (PVA) solution and then mixed with camphene as the porogen. To the best of our knowledge, this is the first study of the production of porous Ti microspheres using a water-in-oil emulsion technique. The primary goal of this study was to fabricate highly porous Ti microspheres with a bioactive surface using a simple alkali treatment.

2. Materials and methods

2.1. Materials and microsphere fabrication

In the current study, Ti powders with a purity of 99.9% and a particle size of <45 μm (Ultimate Materials Technology, Taiwan) were used as the base materials for the elaboration of the porous microspheres. The precursor solutions were prepared as follows. Ti powders were mixed with commercial camphene ($C_{10}H_{16}$, Nan Chung Chemical Co. Ltd., Taiwan), and the weight ratios of camphene to Ti powders (C/T) were 2:1, 4:1, 6:1, 10:1, and 20:1. The camphene was used as the porogen without further purification, and the particle size was less than 550 μm. Porosity and pore size are easily changed by porogen size and loading amount. 0.2 g of gelatin was added into 1.8 ml of deionized water. Using optimal concentration of gelatin can help produce a spherical form of Ti microsphere [19]. The mixture was vigorously stirred and then mixed with the above-mentioned mixture of camphene/Ti. 2 ml of a PVA solution (1% by weight) was added and mixed until homogeneous. Dispersant (PVA solution) was added to produce a stable camphene/Ti/gelatin slurry.

Water-in-oil emulsions, with the above-mentioned precursor solution as the water phase and 60 ml of vegetable oil as the oil phase, were prepared. Emulsions are heterogeneous mixtures of at least one immiscible liquid dispersed in another in the form of droplets [20]. The precursor solution was added into the vegetable oil at 83 °C. The mixed solutions were emulsified for 10 min using magnetic stirring at 650 rpm, which was determined empirically, for preventing particle sedimentation. The mixed solution was dropped into a cool bath, which was approximately 4 °C, where it solidified. The solution was then stirred at 700 rpm for an additional 6 h in order to harden the microspheres. The hardened porous microspheres were ultrasonically cleaned three times in absolute alcohol for 10 min each time and dried for 24 h at 45 °C. Finally, the samples were slowly heated to 210 °C at a rate of 2 °C/min. The temperature was maintained for 3 h to completely burn out the camphene and oil, and then the samples were sintered at 1100 °C in an electric furnace at a heating rate of 10 °C/min for a hold time of 6 h. A vacuum was created inside the furnace, and the atmosphere was exchanged with argon. The procedure for obtaining the Ti microspheres is summarized in Fig. 1.

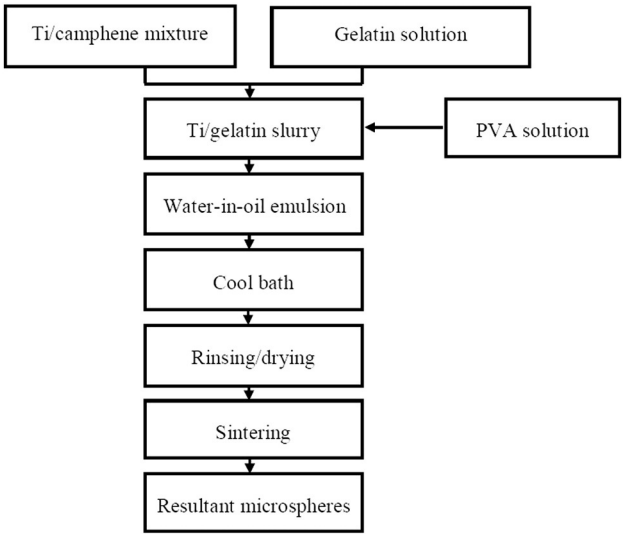

Fig. 1. Procedure for Ti microsphere fabrication.

2.2. Characterization of microspheres

The apparent density of each specimen was calculated from the dry weight and volume. The porosity was then calculated using the equation:

$$\text{Porosity} = (1 - \text{Apparent density}/\rho) \times 100\%$$

where ρ is the theoretical density of the sintered specimens based on a theoretical density for Ti of 4.51 g/cm^3.

Micrographs taken using a scanning electron microscope (SEM; S-3000N, Hitachi, Japan) were used to characterize the cell morphology of the sintered microspheres, and the average pore size was measured from the OM images of the samples prepared by filling the porous Ti microspheres with an epoxy resin. The pore size was estimated using a minimum of 30 pores selected from different places in the cross-sections of three microspheres in each group.

The compressive strength was measured using a desk-top mechanical tester (AG-IS, Shimadzu, Japan) at a crosshead speed of 0.1 mm/min. At least five samples were tested for each condition and the results were averaged. During the compression test, a load was applied until densification of the porous samples started to occur. The compressive strength (MPa) of each specimen was calculated according to $P = 4F/\pi D^2$ [21], where F was the fracture load in N and D the diameter of the specimen in mm.

2.3. Examination of apatite-forming ability

The Ti microspheres were washed with ethanol for 20 min, after which time the samples were cleaned in distilled water for an additional 10 min. The cleaned samples were immersed in 5 M NaOH aqueous

Table 1
Ionic concentrations (mM) of simulated body fluid (SBF) compared to human blood plasma [22].

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HPO_4^{2-}	SO_4^{2-}	HCO_3^-
Blood plasma	142.0	5.0	1.5	2.5	103.0	1.0	0.5	27.0
SBF	142.0	5.0	1.5	2.5	147.8	1.0	0.5	4.2