Biomimetic Apatite Formation on Different Polymeric Microspheres Modified with Calcium Silicate Solutions

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Abstract. Bioactive polymeric microspheres can be produced by pre-coating them with a calcium silicate solution and the subsequent soaking in a simulated body fluid (SBF). Such combination should allow for the development of bioactive microspheres for several applications in the medical field including tissue engineering. In this work, three types of polymeric microspheres with different sizes were used: (i) ethylene-vinyl alcohol co-polymer (20-30 µm), (ii) polyamide 12 (10-30 µm) and (iii) polyamide 12 (300 µm). These microspheres were soaked in a calcium silicate solution at 36.5ºC for different periods of time under several conditions. Afterwards, they were dried in air at 100ºC for 24 hrs. Then, the samples were soaked in SBF for 1, 3 and 7 days. Fourier transformed infrared spectroscopy, thin-film X-ray diffraction, and scanning electron microscopy showed that after the calcium silicate treatment and the subsequent soaking in SBF, the microspheres successfully formed a bonelike apatite layer on their surfaces in SBF within 7 days due to the formation of silanol (Si-OH) groups that are quite effective for apatite formation.

1. Introduction

Biodegradable polymeric microspheres are used for several applications in the biomedical field, namely as carriers of biologically active agents. Furthermore, it is believed that the use of microspheres can lead to more versatile applications in bone repair and might be useful for designing injectable biomaterial systems. When developing new biomaterials for bone regeneration, surface properties must be modulated, ideally in order to mimic the tissue to be replaced. Furthermore, a strong bonding between the host bone and the osteoconductive surface is required. It has been reported that silanol (Si-OH) groups on polymeric materials can induce apatite formation in acellular simulated body fluids (SBF) with ion concentrations nearly equal to those of the human blood plasma [1-3]. Also, it was demonstrated that when an ethylene-vinyl alcohol co-polymer (EVOH) was treated with silane coupling agent and calcium silicate solution and then soaked in SBF a smooth and uniform bonelike apatite layer was able to be formed on the surface of EVOH [4]. The aim of this study is to incorporate Si-OH groups onto polymeric microspheres by soaking them in a calcium silicate solution, in order to obtain bioactive microspheres for bone repair that are able to *in situ* release an antibiotic for treatment and prevention of bone infection.

2. Materials & Methods

Three types of microspheres with different sizes were used as substrates: (i) ethylene-vinyl alcohol co-polymer (EVOH; 20-30 µm), (ii) polyamide 12 (PA 12; 10-30 µm), (iii) polyamide 12 (PA 12; 300 µm). These microspheres were kindly supplied by Trial Corporation, Japan. Tetraethoxysilane (TEOS: $Si(OC_2H_5)_4$), ultra-pure water, ethyl alcohol (C_2H_5OH), 1.0 M-HCl aqueous solution and calcium chloride ($CaCl_2$) were mixed for 10 min at 0ºC in order to prepare calcium silicate solution with a molar ratio of TEOS / H_2O / C_2H_5OH / HCl / $CaCl_2$ of 1.0 / 4.0 or 2.0 / 4.0 or 1.0/ 0.014 / 0.20. The microspheres were immediately soaked in 10 ml of calcium silicate solution with H_2O/TEOS=4/1 at 36.5ºC and 120 rpm for 2 hrs (condition **I**) and soaked for 5 min in a calcium silicate solution with H_2O/TEOS=2/1 prepared 168 hrs before (condition **II**). The specimen were filtered and then dried in air at 100ºC for 24 h. Then, the specimens were soaked in 20 ml of SBF [1] with ion concentrations (Na^+ 142.0, K^+ 5.0, Ca^{2+} 2.5, Mg^{2+} 1.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5 mM) nearly equal to those of the human blood plasma at 36.5ºC and pH = 7.40 for several periods up to 7 days. The specimens were analysed by field-emission scanning electron microscopy (FE-SEM) with an attached energy dispersive electron probe X-ray analyser (EDS), thin-film X-ray diffraction (TF-XRD), and Fourier transform infrared attenuated total reflection spectroscopy (FT-IR). The concentrations of Si, Ca, and P in the SBF solution were measured by inductively coupled plasma atomic emission spectroscopy (ICP) as a function of the immersion time.

3. Results

For both conditions, all the investigated microspheres could be successfully coated with a calcium silicate layer. After the calcium silicate treatment, the reflection peaks of hydroxyl (-OH) and silanol (Si-OH) groups, which are typically observed for a silica gel, and Si-O-Si bond were detected in the FT-IR spectra. The presence of Si-OH groups and Si-O-Si bond suggests that TEOS was successfully hydrolyzed to form Si-OH groups and polycondensed to form Si-O-Si network. The intensity of Si-OH peak was more pronounced for condition **II** than condition **I**.
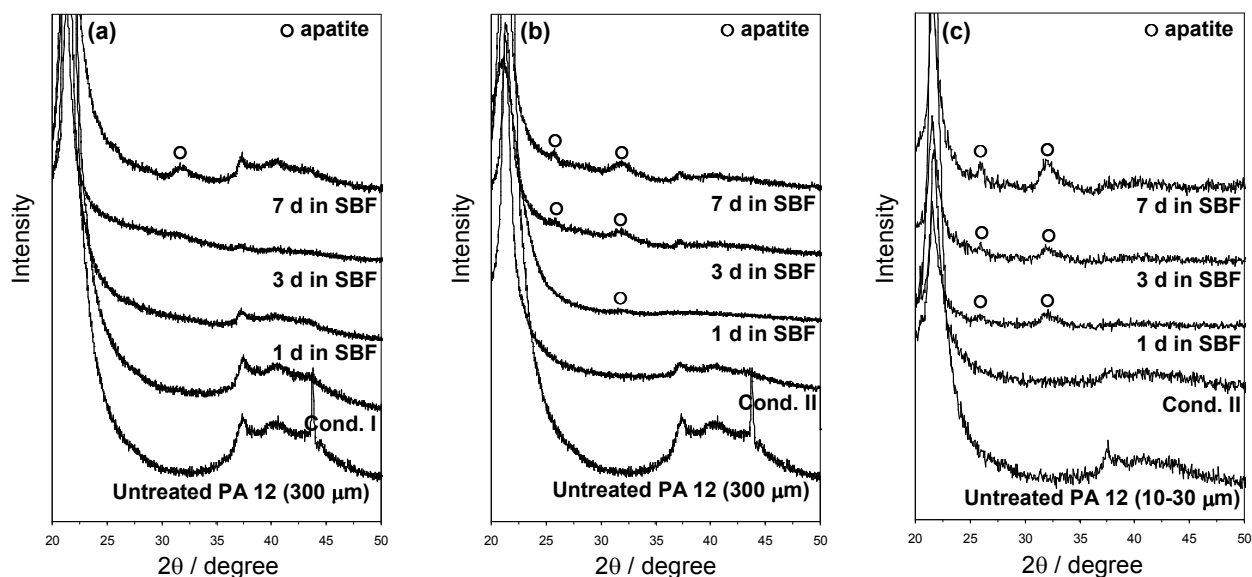
Figure 1 shows TF-XRD patterns of the surfaces of PA 12 (300 µm) and PA 12 (10-30 µm) microspheres, which were coated with a calcium silicate layer under condition **I** or **II** and subsequently soaked in SBF up to 7 days.

Figure 1. TF-XRD patterns of the surfaces of PA 12 (300 µm) and PA 12 (10-30 µm) microspheres, which were coated with a calcium silicate layer under condition **I** or **II** and subsequently soaked in SBF for 1, 3 and 7 days.

For both conditions **I** and **II**, the structure of all microspheres was not changed even after the coating, giving the same diffraction patterns as the untreated microspheres. After soaking in SBF for several periods, the TF-XRD patterns showed several diffraction maxima that can be assigned to an apatite-like phase (ASTM JCPDS 9-432). For condition **II**, all the investigated microspheres showed an ability to induce the formation of an apatite layer within 1 day in SBF (See the Figs. 1-(b) and 1-(c)). However, for condition **I**, only the microspheres PA 12 (300 μm) formed an apatite layer after 7 days in SBF, as shown in Fig. 1-(a). These differences might be attributed to a further progress of hydrolysis and polycondensation of TEOS in the calcium silicate solution due to a long aging period such as 168 hrs in condition **II**. The intensities of apatite peaks increased with increasing soaking time, which indicates the growth of the apatite layer in SBF.

Figure 2 shows FE-SEM images of the surfaces of PA 12 (300 μm) and PA 12 (10-30 μm) microspheres before and after the calcium silicate treatment under condition **I** or **II** and subsequent soaking in SBF up to 7 days.
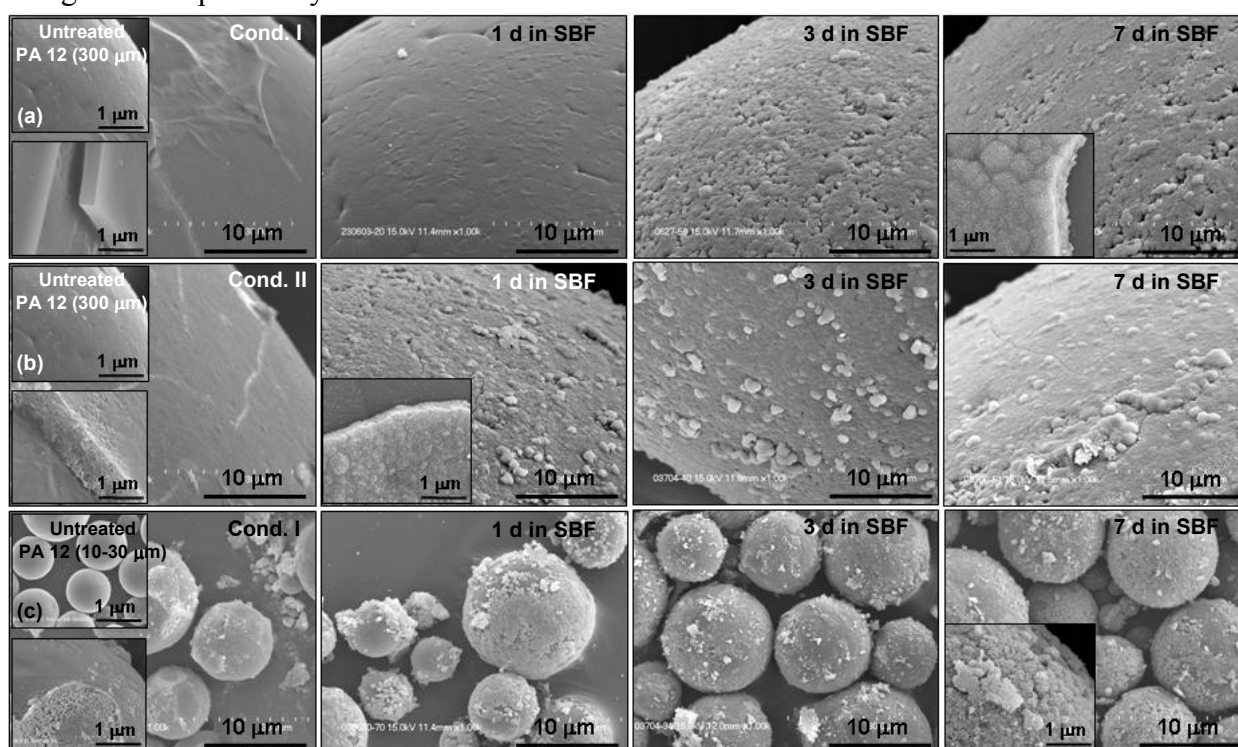

Figure 2. FE-SEM images of the surfaces of PA 12 (300 μm) and PA 12 (10-30 μm) microspheres before and after the calcium silicate treatment under condition **I** or **II** and subsequent soaking in SBF for 1, 3 and 7 days.

For condition **I**, the microspheres PA 12 (300 μm) were completely covered with a uniform apatite layer after 3 days (Fig. 2-(a)). However, this layer was very fine and not dense, which was in agreement with the TF-XRD in Fig. 1-(a). In contrast, when the microspheres PA 12 (10-30 μm) and PA 12 (300 μm) were treated with a calcium silicate solution under condition **II**, all the microspheres were completely covered with a uniform apatite layer within 1 day in SBF as shown in the Figs. 2-(b) and (c). This layer had a finer structure, where needle-like crystals were agglomerated. However, in the case of PA 12 (10-30 μm), the apatite layer was not as dense as for PA 12 (300 μm) (See Figs. 2-(b) and (c)). For EVOH (20-30 μm), the formation of an apatite layer was observed after the calcium silicate treatment in condition **II** and the subsequent soaking in SBF for 1 day. However, an aggregation of these microspheres was found to occur quite easily both in a calcium silicate solution as well as in SBF.

By EDS measurement, an appreciable quantity of Ca and P was detected on the surface of the PA 12 (300 μm) microspheres after the calcium silicate treated under the condition **I** and subsequent

soaking in SBF for 3 days and, in the case of the condition **II**, it was after 1 day in SBF. For PA 12 (10-30 μm) microspheres treated with a calcium silicate solution under condition **II**, the signal of the Ca and P elements was detected after 1 day in SBF, although its intensity was not as high as for PA 12 (300 μm). From the EDS and FT-IR results, it was found that a calcium silicate layer, rich in Ca ions and Si-OH groups, was formed on all the microspheres. However, the intensity of Si signal was larger for the condition **II** than for the condition **I**.

By ICP analysis, for the condition **II**, a decrease in Ca and P concentrations and an appreciable increase in Si concentration were observed. The decrease in the Ca and P concentration is attributed to the formation of an apatite layer by consuming the Ca and P ion from the surrounding fluid. The increase in the Si concentration is attributed to the dissolution of silicate ions from the calcium silicate layer, which results in a formation of the Si-OH groups. These groups are responsible for the apatite nucleation. The amount of these groups can be speculated by the rate of the dissolution of the silica into the SBF, because the dissolution of the silica gel, in this case calcium silicate gel, proceeds via hydrolysis of the silica [2-3].

4. Discussion and Conclusions

It was shown that a calcium silicate layer, rich in Ca ions and Si-OH groups, was able to be formed on the microspheres by the present method and has the ability to induce the formation of a bone-like apatite layer. When the calcium silicate-coated microspheres were soaked in SBF, the Ca ions in the calcium silicate layer were released into SBF to form many Si-OH groups and simultaneously increased the ionic activity product of the surrounding fluid with respect to apatite. The Si-OH groups induced the apatite nucleation, and the increased ionic activity product accelerates the nucleation rate of apatite.

It is noted that there is a compositional difference between the two conditions. Namely, molar ratios of $H_2O/TEOS$ in condition **I** and condition **II** were 4/1 and 2/1, respectively. When the $H_2O/TEOS$ molar ratio of the calcium silicate solution is decreased, the time for gelation decreases [5]. As consequence, the calcium silicate solution in condition **II** gives a higher viscosity to form a uniform calcium silicate layer on the surfaces of the microspheres. Also, the calcium silicate layer obtained in condition **II** had a more porous structure than that in condition **I** (See the Figs. 2-(b) and (a)). It has been demonstrated that the porous structure in the silica gels can be related to the apatite nucleation [6-8]. The calcium silicate layer formed in condition **II** dissolved into SBF more rapidly than that formed in condition **I**, and hence the induction time for apatite formation is reduced.

The resultant microspheres with apatite-forming ability are a very promising material to be used in the bone repair and regeneration field. This work is one of the first reports on the coating of microspheres with biomimetic calcium-phosphate layers.

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