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Vaterite deposition on biodegradable polymer foam scaffolds for inducing bone-like hydroxycarbonate apatite coatings

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Abstract Hydroxycarbonate apatite (HCA) coatings on the surface of bioresorbable materials for bone tissue engineering scaffolds were produced using macroporous poly(DL-lactide) (PDLLA) foams impregnated by calcium carbonate in vaterite crystalline form. Stable and homogeneous vaterite deposition on PDLLA foams was achieved using a slurry dipping technique. In vitro studies in simulated body fluid (SBF) were performed to induce formation of (HCA) on the surface of vaterite/PDLLA composite foams. HCA was detected after immersion of foams in SBF for 7 days. Hence, depositing vaterite on materials followed by immersion in SBF is confirmed to induce HCA coatings on the surface of the material. The HCA coated, bioactive and resorbable PDLLA foams are intended for use as bone tissue engineering scaffolds.

Introduction

Synthetic biodegradable polymers such as poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) have become very important biomaterials for a wide range of applications such as surgical sutures, scaffolds for tissue engineering and controlled drug delivery systems [1–3]. However, a number of problems have been encountered regarding the use of these polymers in tissue engineering applications due to the reported release of acidic degradation products leading to inflammatory responses and to their hydrophobicity leading to poor cell-adhesion [4, 5]. To overcome this limitation, it has been suggested, in particular for bone tissue engineering, that composite scaffolds should be developed, combining these polymers with bioactive inorganic materials such as hydroxyapatite or Bioglass® [6–10]. Such composites have shown controlled degradation behavior, for example in contact with simulated body fluid (SBF), due to the buffering effect of the alkaline inorganic phase [11, 12].

Hydroxycarbonate apatite (HCA) is known to be very similar to the inorganic phase in living bone both in its chemical composition and crystalline structure. Moreover HCA shows high osteoconductivity as well as bioresorbability in biological environments [13, 14]. For these reasons, much attention has been paid to HCA as a biomaterial. In particular, HCA coatings on the surface of synthetic biodegradable polymers such as polylactides are likely to be very attractive for the production of suitable scaffolds for bone tissue engineering.

A preparation method for HCA coatings on different materials based on immersion in SBF, which is a tris-buffer solution with inorganic ion concentrations almost equal to those of human plasma [15], termed ''biomimetic coating method'', is frequently used in the biomaterials field [16]. This method has an advantage over conventional coating techniques in that materials can be homogeneously coated with HCA without the need of a heating process, therefore it is applicable to polymer substrates. Two indispensable

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conditions needed for the formation of HCA coatings on materials using SBF are: (1) the existence of surface functional groups that induce nucleation of HCA and (2) the increase in the supersaturation of the SBF solution.

Generally, no HCA forms on the surface of non-treated synthetic biodegradable polymers such as PLA and PLGA, i.e. on substrates without the presence of surface functional groups [8, 10]. This suggests that incorporating materials with Ca^{2+} ion-releasing ability, in order to increase the supersaturation of Ca in SBF, promotes HCA deposition on the surface of synthetic biodegradable polymers immersion SBF.

Calcium carbonate ($CaCO_3$) has been recognized as a bioresorbable bone filling material and its good osteoconductivity has been confirmed in recent studies [17, 18]. Calcium carbonate is expected to supply both Ca^{2+} and carbonate ions when immersed SBF. As a result, the supersaturation of Ca ions would increase and the concentration of carbonate ions in SBF would come close to that in human blood plasma. It is also expected that the dissolution of calcium carbonate in water would provide a pH buffering effect at the polymer surface. It is well known that calcium carbonate has three polymorphs, viz., calcite, aragonite and vaterite [19]. Solubility of vaterite is higher than that of calcite or aragonite. The authors have already reported that PLA-based composites containing vaterite have much higher HCA-forming ability in SBF than similar composites containing calcite or aragonite without vaterite [20]. It is thus recognized that vaterite is the material of choice to be used in combination with polymers for achieving HCA coating on the surface of polymer materials by the simple immersion method in SBF.

In the present work, HCA coatings on the surface of bioresorbable materials for bone tissue engineering were produced using macroporous poly(DL-lactide) (PDLLA) foams impregnated by vaterite. The PDLLA foams, which exhibit high porosity (> 90%) and oriented pore structure, are being developed for applications as bone tissue engineering scaffolds [21].

Materials and methods

PDLLA foams were fabricated following a thermally induced phase separation (TIPS) process, also termed freeze-drying, which has been described in detail elsewhere [22, 23]. Briefly, 2 g of PDLLA (Purasorb®; Purac biochem, Holland), with inherent viscosity = 1.52 dL/g, was dissolved in 40 mL of dimethylcarbonate (99%, Acros) under magnetic stirring overnight. The solution was transferred into a 150 mL lyophilisation flask and frozen for 2 h by quenching into liquid nitrogen. The flask was then connected to a vacuum pump (10^{-2} Torr) and the solvent was sublimated at –10 °C for the first 48 h, and then at 0 °C for additional 48 h. The residual solvent was then removed at room temperature until the foam reached a constant weight.

Vaterite was prepared using a carbonation process. The suspension was prepared by adding 200 g of $Ca(OH)_2$ and 100 g of distilled water into 2 L of methanol. CO_2 gas was blown for ~2 h at a flow rate of 2 L/min into the suspension at 20 °C. A gelation occurred by introducing CO_2 gas into the suspension. After gelation, the obtained particles were gathered by filtration and dried at 110 °C to prepare fine-sized powders. The BET surface area was measured to be ~70 m^2/g.

Pre-treatment of the PDLLA foams with ethanol was carried out at room temperature for 1 h, following a procedure originally described by Mikos et al [24]. The same pretreatment procedure has been used to coat PDLLA foams with Bioglass® particles [25]. Vaterite deposition on foams was prepared by a dipping process. For preparation of the dipping suspensions; 1 wt% of the vaterite powder was added either to methanol or ethanol. An alcohol was used as a solvent for preventing the fast dissolution of vaterite in water. The concentration of the slurry was determined by an optimization process based on a trial-and-error approach to achieve satisfactory results in terms of structural stability of the coating and adequate infiltration of the vaterite particles into the pores of the foams. The suspensions were stirred for 10 min before immersion of the foams. The pre-treated PDLLA foams were dipped into the prepared slurry for 3 min. The samples were withdrawn at a velocity of 5 cm/s, and subsequently dried at room temperature in air atmosphere for 24 h and stored in desiccators for further studies.

The vaterite/PDLLA composite foams were soaked at 37 °C in SBF consisting of 2.5 mM of Ca^{2+}, 142.0 mM of Na^+, 1.5 mM of Mg^{2+}, 5.0 mM of K^+, 148.8 mM of Cl^-, 4.2 mM of HCO_3^-, 1.0 mM of HPO_4^{2-}, and 0.5 mM of SO_4^{2-}. The SBF solution also included 50 mM of $(CH_2OH)_3CNH_2$ and 45.0 mM of HCl at pH 7.4. After removal from the SBF solution, the samples were gently washed with distilled water and then dried in air atmosphere at room temperature. The crystalline phases in vaterite/PDLLA foams were identified by XRD, and the morphology of the porosity as well as the pore size, before and after SBF immersion, were characterized by SEM (JEOL; JSM-T220).

Results and discussion

Figure 1 shows an X-ray diffraction (XRD) pattern and a scanning electron microscopy (SEM) image of the powders. XRD analysis shows that the calcium carbonate powder consists predominantly of vaterite with small

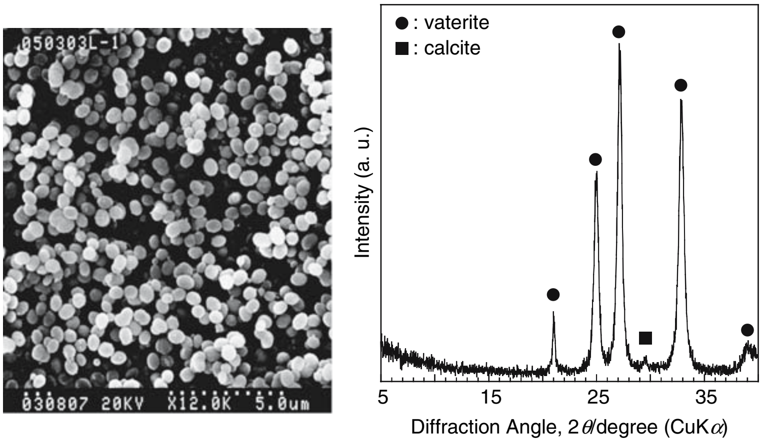

Fig. 1 SEM image and XRD pattern of as-prepared vaterite

amounts of calcite. The SEM image shows that the vaterite particles, which have a tendency to form in fine-sized spherical shape [26], are on average $\lesssim$0.5 μm.

Figure 2a, b shows XRD patterns and SEM images of vaterite/PDLLA composite foams prepared using methanol and ethanol slurries, respectively. XRD patterns show that the peaks corresponding to vaterite are seen in both samples. This suggests that the medium chosen for preparation of the slurry has no effect on the crystal phase of dispersed vaterite particles after slurry dipping. It can be observed however that the amount of vaterite deposition on PDLLA foams prepared using the methanol slurry is higher than that on PDLLA foams prepared using the ethanol slurry. Thus, the following experiments were carried on foams prepared using methanol based slurries. This implies that the affinity of vaterite to methanol solvent is better than that to ethanol. Vaterite slurries based on methanol showed a lower viscosity due to good dispersibility, compared with ethanol slurries. As a result, a large amount of vaterite particles was deposited on the surfaces of foam struts during the dipping procedure using the methanol slurry. This is confirmed by observing SEM images of vaterite/PDLLA composite foams obtained at high magnification, prepared using methanol and ethanol slurries, respectively, as shown in Fig. 3a, b. SEM images show that vaterite particles of ~0.5 μm in diameter are homogeneously dispersed on the surface of the foam struts. It should be highlighted that some tiny pores of ~1 μm in diameter are observed on surfaces of struts of foams prepared using methanol slurries. It is thought that these pores are formed by initial hydrolyzation of PDLLA due to a large amount of deposited vaterite particles.

Figure 4 shows XRD patterns of vaterite/PDLLA composite foams after immersion in SBF. Results show that after 1 day of immersion, the peak corresponding to vaterite disappears. After 7 days of immersion, new peaks at around $2\theta \sim 29°$ due to formation of calcite and at around $2\theta \sim 32°$ due to formation of HCA are detected. It is

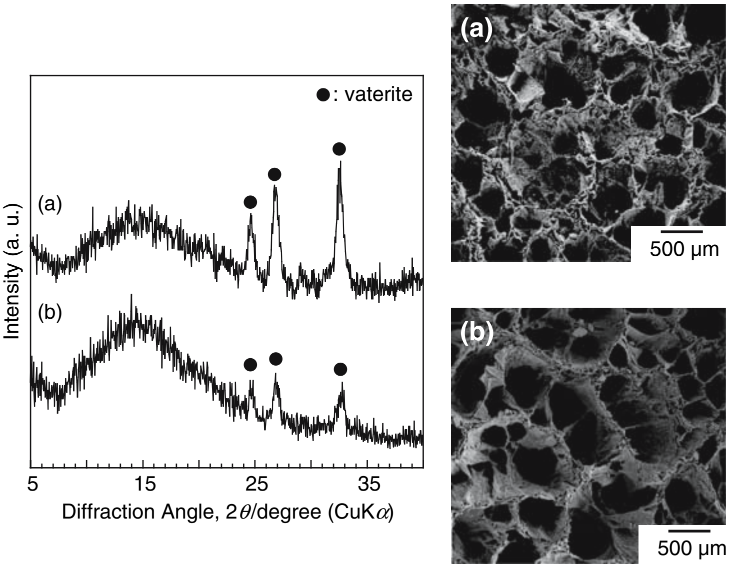

Fig. 2 XRD patterns and SEM images of vaterite/PDLLA composite foams prepared using (**a**) methanol or (**b**) ethanol slurries

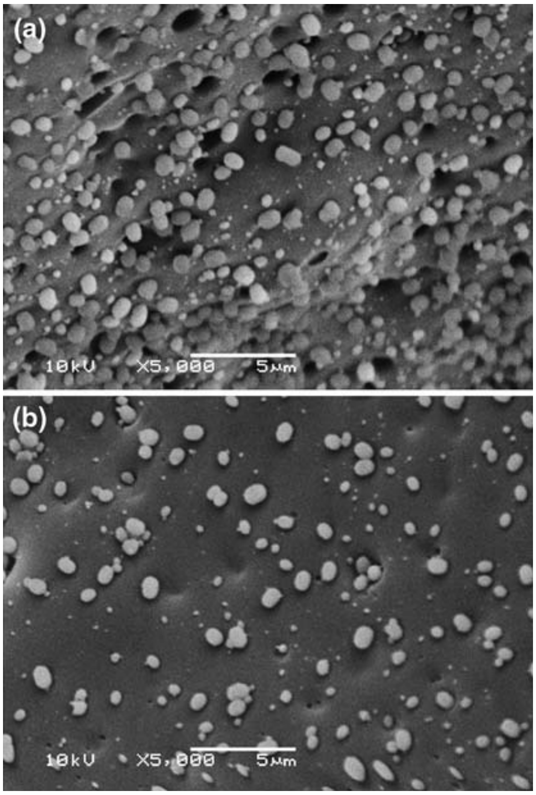

Fig. 3 High magnification SEM images showing high density of vaterite particles deposited on foams prepared using (**a**) methanol or (**b**) ethanol slurries

suggested that a large amount of Ca^{2+} ions are rapidly released by the decomposition of nano-sized vaterite particles after soaking the composite in SBF. As a result, HCA and calcite form on the surfaces of the foam struts utilizing excessive Ca^{2+} ions from the vaterite phase. Figure 5a–c shows three different SEM images of vaterite/PDLLA composite foams after immersion in SBF for 7 days, at different magnification. As shown on the micrographs, the surface of the foam struts is covered with a relatively homogeneous deposit, which is identified as the HCA or calcite phases according to the XRD results shown in Fig. 4. A calcite phase will inevitable form after immersion under the present conditions. The newly formed calcite is nevertheless assumed to be of advantage for bone repair strategies because of the good osteoconductivity of this ceramic phase. The formation of HCA on polymer surfaces immersed in SBF has been shown to occur for example if Bioglass® or hydroxyapatite are added to the polymer to impart bioactivity [7, 8, 11]. Boccaccini et al. reported that HCA formed on PDLLA/Bioglass® composite foams fabricated using slurry dipping technique after 7 days of immersion in SBF [25]. In similar experiments, Francis et al. [27] reported that after 7 days of immersion in SBF, PDLLA/Bioglass® composite foams fabricated using TIPS develop HCA on their surfaces. Thus, vaterite/PDLLA composite foams have the same HCA forming ability as PDLLA/Bioglass® composite foams presented previously in the literature [26, 27]. It is therefore suggested that vaterite could be considered as an alternative second phase, such as Bioglass® or hydroxyapatite, in PDLLA composite foams for bone tissue engineering. The advantage of vaterite for use in biodegradable scaffolds is that it completely dissolves in contact with aqueous media at a much faster rate than Bioglass® or hydroxyapatite. Therefore, when vaterite/PDLLA composite foams are immersed in SBF, vaterite completely dissolves and HCA directly forms on the PDLLA foam surface without intermediate layers forming on the polymer surfaces. It is expected that the polymer/HCA interface prepared after immersion in SBF using vaterite/PDLLA composite foams will be more stable and will exhibit greater adhesion, compared to that in the PDLLA/Bioglass® system. However several advantages of Bioglass®, in particular its osteoproduction effect [28], is not exhibited by vaterite, therefore the smart combination of PDLLA with Bioglass® and vaterite additions should be tailored for particular applications.

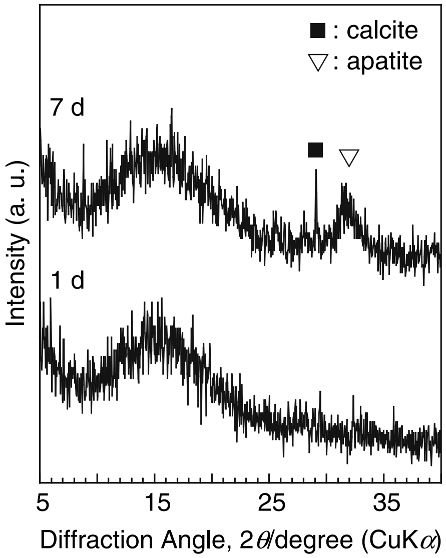

Fig. 4 XRD patterns of vaterite/PDLLA composite foams prepared using methanol slurry dipping process, after immersion in SBF for 1 and 7 days, showing formation of calcite and apatite

Conclusion

Vaterite/PDLLA composite foams were fabricated by a slurry dipping technique using an alcohol as a solvent. The optimized slurry dipping technique led to stable and uniform vaterite deposition on the surface of the foam struts. PDLLA foams covered with a bone-like HCA layer were prepared by immersion of the vaterite/PDLLA composite

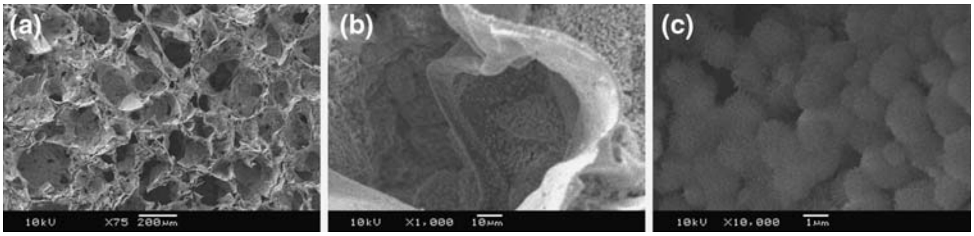

Fig. 5 SEM images of vaterite / PDLLA composite foams prepared using ethanol slurry dipping process, after immersion in SBF for 7 days showing formation of the HCA layer at (**a**) low, (**b**) medium and (**c**) high magnification

foams in SBF. It was found that a homogeneous HCA layer developed after 7 days of immersion in SBF. Vaterite deposition is thus a promising approach to achieve homogeneous HCA coating on the surface of polymer materials by the simple immersion method in SBF. The 3-dimensional HCA coated PDLLA foams developed in this study represent attractive bioactive materials for fabrication of scaffolds for bone tissue engineering.

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