

Research Article

Preparation and In Vitro Behavior of a Poly(lactic acid)-Fiber/Hydroxyapatite Composite Sheet

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This paper describes the processing and in vitro behavior of a poly(lactic acid) (PLA)-fiber/hydroxyapatite (HA) composite sheet consisting of a knitted PLA-fiber sheet and HA powder for bone tissue engineering. Type I collagen was used as a binding agent to combine the PLA fibers and the HA powder. Precipitate formation in Hanks' balanced salt (HBS) solution was monitored to evaluate the in vitro apatite formation ability of the PLA-fiber/HA composite sheet. Precipitate formation was observed on the surface of the PLA-fiber/HA composite sheet after immersion in HBS solution for only 1 day, while no precipitate formation was observed on the PLA-fiber sheet without HA as a control. In conclusion, a PLA-fiber/HA composite sheet for use as a scaffold was successfully prepared. Within the limitations of this investigation, we confirmed that the PLA-fiber/HA composite sheet has a high apatite formation activity compared with the PLA-fiber sheet and represents a promising material for use as a scaffold.

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1. Introduction

Hydroxyapatite (HA) $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ and related calcium phosphates are widely used as bone substitutes or scaffolds for bone tissue engineering because of their good bioactivities and osteoconductivities. In general, HA can take on many forms, including powder, granule, pellet, and porous block formations. However, the brittleness and insufficient strength of HA restrict its applications to physiologically nonload-bearing bone lesions. To overcome these shortcomings of HA, composites of HA and biodegradable polymers with high flexibility have been prepared as scaffold materials for bone tissue engineering [1, 2]. Jansen et al. [3] prepared a new biodegradable membrane material comprised of poly(ethylene glycol terephthalate)/poly(butylene terephthalate) coated with HA powder and found good properties for use in guided tissue regeneration membranes. Therefore, the brittleness of HA ceramics can be improved by combination with tough biodegradable polymers. Owing to its biodegradability and biocompatibility, poly(lactic acid)

(PLA) is one of the main biodegradable polymer groups used in biomaterial research to create carriers for drug delivery systems, scaffolds for tissue engineering, and implanted medical devices [4, 5]. In particular, the fibrous forms of PLA, such as knitted and woven fabrics, are more preferable than the bulk material for bone tissue engineering, because these forms are highly porous and thus encourage the migration and adhesion of osteoblast-like cells. With these backgrounds, the development of composites of PLA fibers and HA is attractive, since the advantageous properties of these two types of materials can be combined to better suit the biological and mechanical demands for biomedical uses.

In this study, a knitted PLA-fiber/HA composite sheet was prepared for bone tissue engineering. In general, apatite precipitation in biological fluids is an important determinant for the bioactivity of a PLA-fiber/HA composite sheet. Therefore, we examined the precipitate formation on the surface of the PLA-fiber/HA composite sheet in a simulated body fluid.

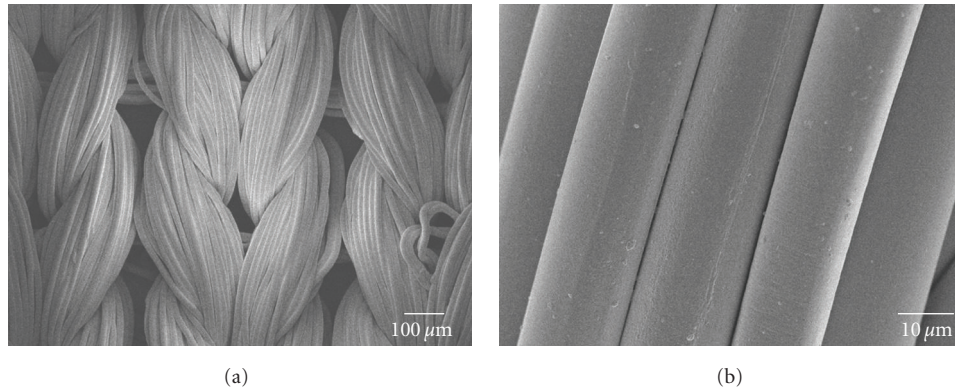


FIGURE 1: FE-SEM images of the PLA-fiber sheet used. (a) Magnification $\times 100$. (b) Magnification $\times 2,000$.

2. Materials and Methods

2.1. Materials and Preparation. First, HA powder (HAP-100; Taihei Chemical Industries, Osaka, Japan) and 1.0 wt% of pig hide type I collagen solution (Nippon Meat Packers Inc., Osaka, Japan) were combined in an HA-collagen slurry preparation. The slurry of HA-collagen composites was prepared with an HA: collagen mixing ratio of 1 : 2, using a planetary ball mill (P5/2; Fritsch Japan Co. Ltd., Kanagawa, Japan) for 30 minutes. The resulting slurry was poured into a stainless mold (ϕ 50 mm), followed by the addition of 1 mL HEPES buffer solution (Gibco, Tokyo, Japan) for 9 mL HA-collagen slurry. A knitted PLA-fiber sheet (Corefront Corp., Tokyo, Japan) (Figure 1) which was trimmed to the same size as the mold (ϕ 50 mm) was dipped into the HA-collagen slurry. To allow gelation, the HA-collagen slurry containing the PLA-fiber sheet was stored at 37°C for 24 hours. Subsequently, the HA-collagen gel containing the PLA-fiber sheet was frozen at -80°C for 48 hours and dried in a freeze-drying machine (FDU-1200; EYELA Co. Ltd., Tokyo, Japan) for 48 hours. Finally, the composite of the PLA-fiber sheet with HA (PLA-fiber/HA composite sheet) was removed from the mold. Figure 2 shows a typical PLA-fiber/HA composite sheet fabricated in this study.

2.2. Observation by Field-Emission Scanning Electron Microscopy. Vacuum drying and platinum sputtering of the specimen surface were carried out. The appearance of the PLA-fiber/HA composite sheet was observed under a field-emission scanning electron microscope (FE-SEM) (JSM-6340F; JEOL, Tokyo, Japan) at an acceleration voltage of 5 kV.

2.3. Thin-Film X-Ray Diffraction. The PLA-fiber/HA composite sheet was characterized by thin-film X-ray diffraction (TF-XRD) (XRD-6100; Shimadzu, Kyoto, Japan). The X-ray source was Cu, the power was 40 kV \times 30 mA, and the scanning range was 20–40°.

2.4. Immersion in Simulated Body Fluid. PLA-fiber/HA composite sheets (10 mm long \times 10 mm wide) were immersed in

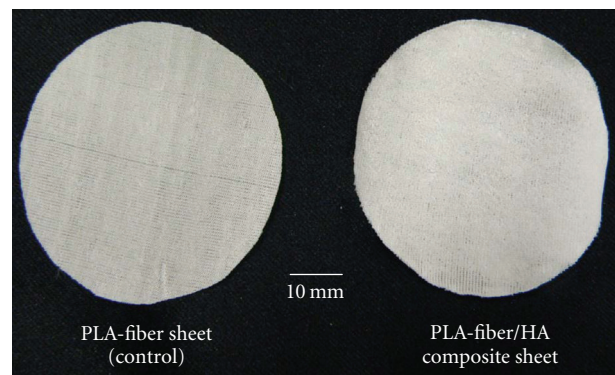


FIGURE 2: Photograph of the fabricated PLA-fiber/HA composite sheet.

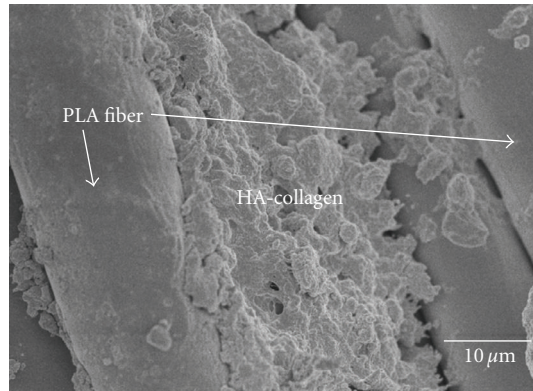
20 mL of simulated body fluid, comprising Hanks' balanced salt (HBS) solution without organic species (pH = 7.4) [6], at 37°C in a Teflon-sealed polystyrene bottle for 1 or 7 days. The composition of the HBS solution used is shown in Table 1. The solution and bottle were changed every day, to expose the specimens to fresh solution. After immersion in the HBS solution for 1 or 7 days, the PLA-fiber/HA composite sheets were rinsed with double-distilled water to remove the HBS solution and then immediately dried in a vacuum desiccator. The morphology of the precipitates on the PLA-fiber/HA composite sheet surface was observed under an FE-SEM at an acceleration voltage of 5 kV.

3. Results and Discussion

Figure 3 shows an FE-SEM image of a fabricated PLA-fiber/HA composite sheet. Tight binding between the PLA fibers and HA mediated by collagen was observed in the PLA-fiber/HA composite sheet. Collagen is widely used in biomaterials because hard tissues such as bone and teeth are composed of HA and collagen [7, 8]. Additionally, several studies have successfully prepared HA/collagen/PLA or HA/collagen/poly(lactic-co-glycolic acid) (PLGA) composites [9, 10]. Therefore, in the present study, collagen was used as a binding agent to combine the PLA fibers and HA

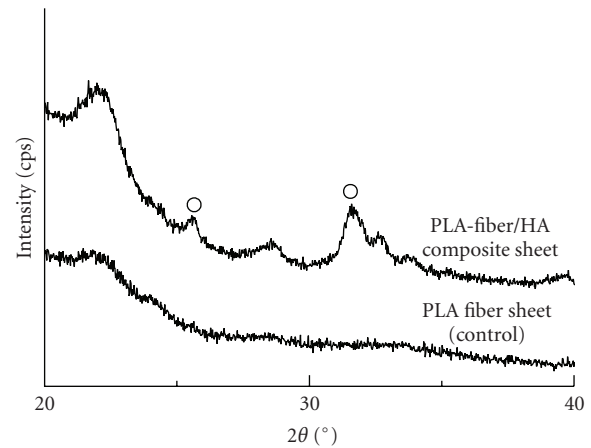
TABLE 1: Inorganic ion concentrations in the HBS solution.

Ion	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	HPO ₄ ²⁻	SO ₄ ²⁻	HCO ₃ ⁻
Concentration (mmol/L)	142.	5.81	0.811	1.26	145.	0.788	0.811	4.17

FIGURE 3: FE-SEM image of the PLA-fiber/HA composite sheet. (Original magnification $\times 2,000$).

powder in the PLA-fiber/HA composite sheet. As a result, the multifilament PLA fibers were thoroughly infiltrated with the HA-collagen slurry using a dipping method. Furthermore, the PLA-fiber/HA composite sheet exhibited similar flexibility to the PLA-fiber sheet without HA. Figure 4 shows the TF-XRD spectra of the PLA-fiber/HA composite sheet and PLA-fiber sheet. The spectrum of the PLA-fiber/HA composite sheet exhibited peaks at 2θ values of 25.56 and 31.60 degrees, which can be assigned to apatite. This means that the HA powder was homogeneously dispersed on the surface of the PLA fibers in the PLA-fiber/HA composite sheet.

As an *in vitro* biocompatibility evaluation, the influences of PLA-fiber sheets with or without HA were investigated by monitoring the apatite formation in HBS solution. Figure 5 shows a series of FE-SEM photographs revealing the surface appearances of PLA-fiber/HA composite sheets after immersion in HBS solution for 1 or 7 days. After 1 day of immersion, no precipitate formation was observed on the PLA-fiber sheet without HA as a control. In contrast, the formation of precipitated globules was observed on the PLA-fiber/HA composite sheet after 1 day of immersion. The surfaces of both the PLA-fiber/HA composite sheet and the PLA-fiber sheet were completely covered with globules after 7 days of immersion. Pereira [11] suggested that simulated body fluid may induce spontaneous precipitation of HA owing to the release of calcium ions from the material surface. Mochizuki et al. [12] reported that the faster degradation of HA/PLGA composites provides greater degrees of new bone formation. In particular, the enrichment of calcium and phosphorous ions in the microenvironment seems to promote apatite formation. In the present study, the apatite formation ability of the PLA-fiber/HA composite sheet showed positive responses compared with the pure PLA-fiber sheet as a control. The results of the present



○ Apatite

FIGURE 4: TF-XRD patterns of the PLA-fiber/HA composite sheet and PLA-fiber sheet. The spectrum of the PLA-fiber/HA composite sheet exhibits apatite peaks at 2θ values of 25.56° and 31.60°.

study suggest that the release of calcium ions from the PLA-fiber/HA composite sheet was the major contributor to the precipitation of apatite and served as an initiation process for the precipitation of apatite. In our previous study, *in vivo* bone formation on sintered tricalcium phosphate sheets confirmed the results of *in vitro* simulated body fluid immersion experiments, in that more apatite formation corresponded with better bone formation [13]. Therefore, the *in vivo* bioactivity, or osteoconductive ability, of a biomaterial is precisely mirrored by the *in vitro* apatite formation ability in simulated body fluid. On that basis, the present PLA-fiber/HA composite sheet is predicted to have a higher osteoconductive property than the PLA-fiber sheet. The biological activities of the PLA-fiber/HA composite sheet such as cultured cell growth and tissue responses will be investigated in future studies.

Finally, composites reinforced by the nanofibers have several advantages as scaffolds for bone tissue engineering compared with composites by microfibers, due to their high specific surface area and porous structure. The fabrication and characterization of nanofibrous composite scaffolds will be investigated in our next series of experiments.

4. Conclusions

In this study, a PLA-fiber/HA composite sheet for use as a scaffold was successfully prepared. Within the limitations of this investigation, we confirmed that the PLA-fiber/HA composite sheet had high apatite activity compared with the PLA-fiber sheet and represented a promising material for use as a scaffold.

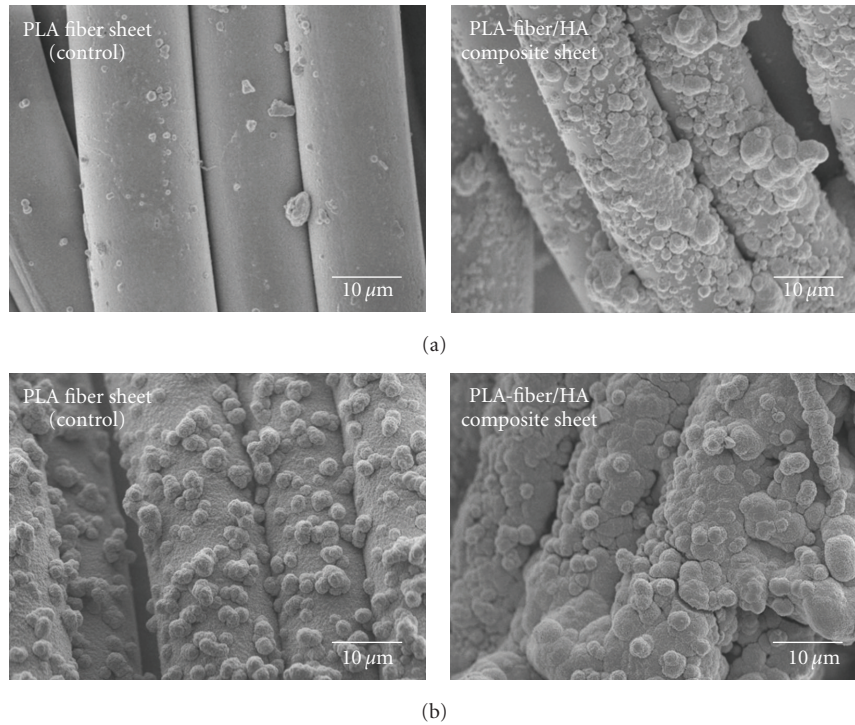


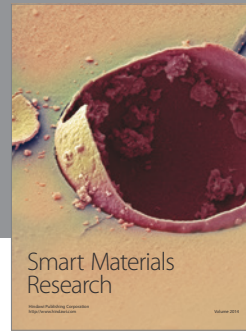
FIGURE 5: FE-SEM photographs showing the surface appearances of the PLA-fiber/HA composite sheet and PLA-fiber sheet after immersion in HBS solution. (Original magnification $\times 2,000$). (a) After 1 day of immersion. (b) After 7 days of immersion. Precipitate formation is observed on the surface of the PLA-fiber/HA composite sheet after immersion in HBS solution for only 1 day.

Acknowledgment

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