Hindawi Publishing Corporation
Advances in Materials Science and Engineering
Volume 2013, Article ID 140643, 6 pages
http://dx.doi.org/10.1155/2013/140643

Research Article

Morphology and *In Vitro* Behavior of Electrospun Fibrous Poly(D,L-lactic acid) for Biomedical Applications

Toshihiro Inami,[1] Yasuhiro Tanimoto,[2] Masayuki Ueda,[1] Yo Shibata,[3] Satoshi Hirayama,[4] Masaru Yamaguchi,[1] and Kazutaka Kasai[1]

[1] *Department of Orthodontics, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho Nishi, Matsudo, Chiba 271-8587, Japan*
[2] *Department of Dental Biomaterials, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho Nishi, Matsudo, Chiba 271-8587, Japan*
[3] *Department of Oral Biomaterials and Technology, Showa University School of Dentistry, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan*
[4] *Department of Dental Caries and Aesthetic Dentistry, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho Nishi, Matsudo, Chiba 271-8587, Japan*

Correspondence should be addressed to Yasuhiro Tanimoto; tanimoto.yasuhiro@nihon-u.ac.jp

Received 26 December 2012; Accepted 10 May 2013

Academic Editor: Peter Majewski

This work describes the fabrication, optimization, and characterization of electrospun fibrous poly(D,L-lactic acid) (PDLLA) for biomedical applications. The influences of the polymer concentration of the electrospinning solution (5, 10, or 15 wt%) and the solution flow rate (0.1, 0.5, 1.0, or 2.0 mL/h) on the morphology of the obtained fibrous PDLLA were evaluated. The *in vitro* biocompatibility of two types of PDLLA, ester terminated PDLLA (PDLLA-R) and carboxyl terminated PDLLA (PDLLA-COOH), was evaluated by monitoring apatite formation on samples immersed in Hanks' balanced salt (HBS) solution. 15 wt% polymer solution was the most beneficial for preparing a fibrous PDLLA structure. Meanwhile, no differences in morphology were observed for PDLLA prepared at various flow rates. Apatite precipitate is formed on both types of PDLLA only 1 day after immersion in HBS solution. After 7 days of immersion, PDLLA-COOH showed greater apatite formation ability compared with that of PDLLA-R, as measured by thin-film X-ray diffraction. The results indicated that the carboxyl group is effective for apatite precipitation in the body environment.

1. Introduction

Owing to its biodegradability and biocompatibility, poly(lactic acid) (PLA) has been demonstrated to be a suitable biodegradable polymer to produce carriers for drug delivery systems, scaffolds for tissue engineering, and implanted medical devices [1–3]. In particular, the fibrous form of PLA is more preferable than the bulk material for bone tissue engineering, because its high porosity encourages the migration and adhesion of osteoblast-like cells [4].

Electrospinning is a simple and effective method for the production of fibrous structures from polymer solutions [5–7]. The advantage of electrospinning method is that it can be used to produce fibrous structures having fiber diameters ranging from a few micrometers to a few hundred nanometers, with large specific surface areas. A schematic diagram of the electrospinning method is shown in Figure 1. There are basically three components required for the process: a high voltage supply, a capillary tube with a pipette or needle of small diameter, and a metallic collecting screen. In electrospinning, a polymer solution is converted to a fibrous structure via electrostatic forces. The polymer solution is delivered through a needle and a high voltage is applied to induce charges in the fluid. When the applied electric field is strong enough to overcome the surface tension, a tiny jet is ejected from the surface of the droplet and drawn toward the collecting plate. Between the needle tip and the collector, the jet is highly stretched, dried, and deposited as

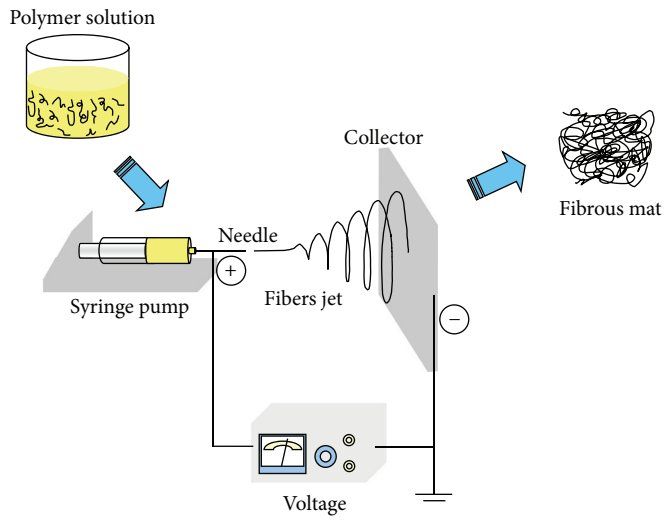

Figure 1: Schematic illustration of the electrospinning apparatus.

a nonwoven fiber structure onto the collector. This process is affected by the polymer characteristics, polymer solution parameters, and process parameters. By varying these parameters it is possible to obtain fibrous structures with various morphologies and properties. For example, Hiep and Lee [8] fabricated an electrospun poly(lactic-co-glycolic acid) (PLGA)/polycaprolactone (PCL) blend using various percentages of PLGA in blended PLGA/PCL solutions. The authors concluded that increasing the PLGA concentration affected the mechanical properties of the electrospun membranes and increased the biocompatibility of the resulting fibrous PLGA/PCL mats. Likewise, Ngiam et al. [9] prepared various types of nanofibers, such as pure PLGA and blended PLGA/collagen nanofibers, by electrospinning and investigated their biomineralization ability for nanohydroxyapatite (n-HA) using an alternate calcium phosphate dipping method. The authors reported preferential deposition of n-HA crystallites on the PLGA/collagen nanofibers compared with those on the PLGA nanofibers, suggesting that collagen is a good template material for n-HA nucleation.

The *in vivo* bioactivity, or osteoconductive ability, of a biomaterial is precisely mirrored by its *in vitro* apatite formation ability in simulated body fluid [10]. In particular, there have been several reports that carboxyl (COOH) groups play an effective role in apatite nucleation in the body environment [11–13]. Kitahara et al. [14] prepared three-dimensional (3D) porous PLA scaffolds by freeze-drying and introduced COOH onto their surface, reporting subsequent improvement of the PLA biomaterial for effective and efficient bone regeneration.

With these backgrounds, fibrous PLA with COOH-terminus prepared by electrospinning is considered to be an effective material for apatite formation ability and bone regeneration, because of its high specific surface area and porous structure. However, little is known about biological activity of electrospun fibrous PLA with COOH-terminus.

In the present study, fibrous poly(D,L-lactic acid) (PDLLA) fabricated by electrospinning for biomedical applications was optimized and characterized. Moreover, as an evaluation of *in vitro* biocompatibility, the influence of fibrous PDLLA with or without COOH-terminus on apatite formation in simulated body fluid was investigated.

2. Materials and Methods

2.1. Materials. In this study, two types of PDLLA, ester terminated PDLLA (PDLLA-R) with an inherent viscosity of 0.37 dL/g and carboxyl terminated PDLLA (PDLLA-COOH) with an inherent viscosity of 0.32 dL/g, were supplied by DURECT Corporation, AL, USA. Both PDLLA-R and PDLLA-COOH were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Wako, Osaka, Japan) at concentrations of 5, 10, and 15 wt% at room temperature (RT) overnight until the solutions became homogeneous.

2.2. Electrospinning. As mentioned in Figure 1, the electrospinning set-up (IMC-1639, Imoto, Kyoto, Japan) consisted of a high voltage power supply (Matsusada Precision Inc., Shiga, Japan), a syringe pump (KDS-100, ISIS Co. Ltd., Osaka, Japan) with needle, and a stainless steel collector plate. The solution was loaded into a 30 mL plastic syringe (Terumo Corp., Tokyo, Japan) having a 21G stainless steel needle (Terumo Corp.). The PDLLA solution was continuously supplied at flow rates of 0.1, 0.5, 1.0, and 2.0 mL/h through the needle using a syringe pump. The distance between the needle tip and the stainless steel collector was 10 cm. Upon application of a high voltage of 15 kV between the needle tip and the collector, the PDLLA solution was evaporated, and fibrous PDLLAs were deposited on the collector plate at RT. The obtained fibrous PDLLA was vacuum dried overnight at RT.

2.3. Field-Emission Scanning Electron Microscopy. Vacuum drying and platinum sputtering of the specimen surface were carried out, and the appearance of the fibrous PDLLA was then observed under a field-emission scanning electron

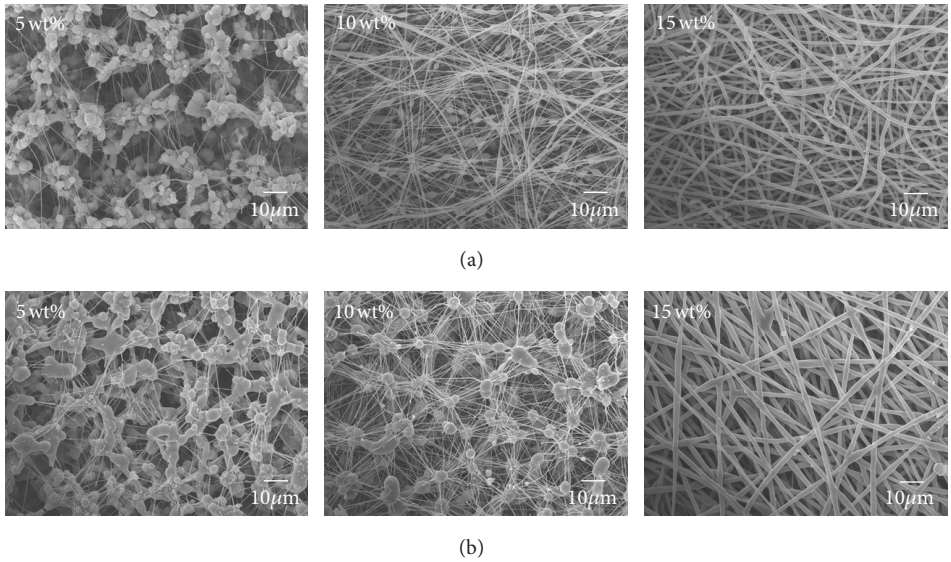

Figure 2: FE-SEM images showing the morphology of fibrous PDLLA prepared with varying solution concentrations. (a) PDLLA-R; (b) PDLLA-COOH.

Table 1: Concentrations of inorganic ions 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

microscope (FE-SEM; JSM-6340F, JEOL, Tokyo, Japan) at an accelerating voltage of 5 kV.

2.4. Characterization of Calcium Phosphate Formed on Fibers in Body Simulated Fluid. PDLLA (10 mm × 10 mm) samples were immersed in 20 mL of simulated body fluid, comprising Hanks' balanced salt (HBS) solution without organic species (pH = 7.4) [15], 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 fibrous PDLLAs were rinsed with double-distilled water and then immediately dried in a vacuum desiccator. The morphology of the precipitates on the PDLLA surface was observed by FE-SEM at an accelerating voltage of 5 kV. The precipitates were also characterized by thin-film X-ray diffraction (TF-XRD; RINT2000, Rigaku Corp., Tokyo, Japan) using a Cu Kα X-ray source, power of 50 kV × 100 mA, and scanning range of 20–40°.

3. Results and Discussion

Electrospinning is a versatile polymer processing technique in which a stream of polymer solution or melt is subjected to a high electric field, resulting in formation of a fibrous structure. In the present study, fibrous PDLLAs were prepared by electrospinning for biomedical applications.

The morphology of fibrous materials fabricated by electrospinning is dependent on variables such as fabrication parameters and composition [16, 17]. First, the influence of the solution concentration on the morphology of the electrospun fibrous PDLLA was evaluated. Figure 2 shows FE-SEM images of the structures of fibrous PDLLAs prepared from solution concentrations of 5, 10, and 15 wt% at a flow rate of 2.0 mL/h. In general, fiber morphology varied with PDLLA solution concentration. At a concentration of 5 wt%, many beads or beaded bulks were observed in both PDLLA-R and PDLLA-COOH. At 10 wt%, numerous beads or beaded fibers connected together were still observed throughout the material in both PDLLA-R and PDLLA-COOH. In contrast, fibrous structures were observed for both PDLLA-R and PDLLA-COOH at a concentration of 15 wt%, and the beads completely disappeared. Generally, the amount of the beads decreases with increasing polymer concentration [17]. The shape of the PDLLA changed from bead to fiber when the polymer concentration was increased from 10 to 15 wt%. Thus, it was suggested that the optimum solution concentration for producing fibrous PDLLA was 15 wt%. Next, the influence of the flow rate on morphology of fibrous PDLLA electrospun from 15 wt% solution was evaluated. Figure 3 shows FE-SEM images of the structures of the obtained fibrous PDLLA for flow rates of 0.1, 0.5, and 1.0 mL/h. As previously mentioned, the structure of PDLLA prepared with a solution concentration of 15 wt% at rate of 2.0 mL/h is shown in Figure 2. No large differences in microstructure were found between the samples prepared with flow rates of 0.1, 0.5, 1.0, and 2.0 mL/h.

Apatite precipitation in biological fluids is an important determinant of the bioactivity of fibrous PDLLA. Therefore, we examined precipitate formation on the surface of the obtained PDLLA in HBS solution. Figure 4 shows FE-SEM images of the surface appearances of fibrous PDLLA samples

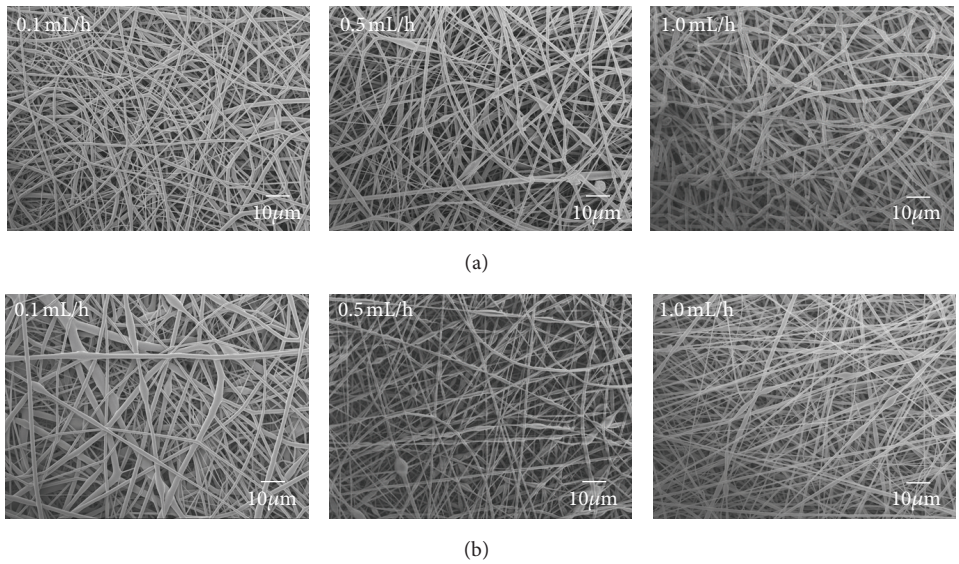

Figure 3: FE-SEM images showing the morphology of fibrous PDLLA prepared with varying flow rates. (a) PDLLA-R; (b) PDLLA-COOH.

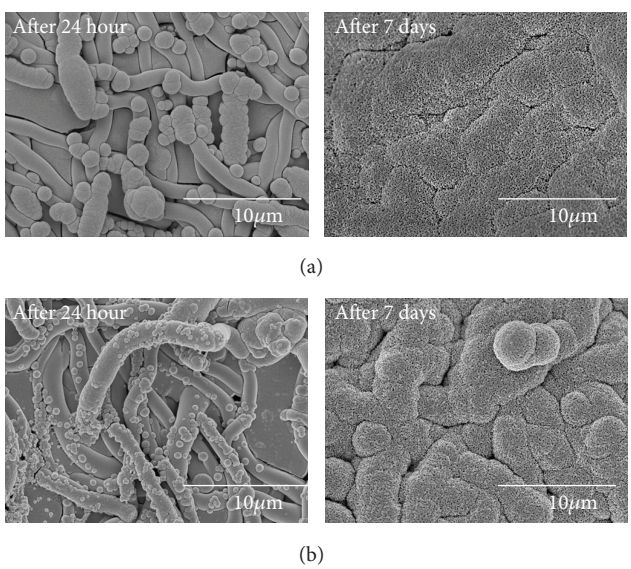

Figure 4: FE-SEM photographs showing the surface appearances of the fibrous PDLLA after immersion in HBS solution. (Original magnification 5,000x.) (a) PDLLA-R; (b) PDLLA-COOH.

after immersion in HBS solution for 1 or 7 days. Precipitated small globules were observed on both the fibrous PDLLA-R and PDLLA-COOH after 1-day immersion. The surfaces of both the PDLLA-R and the PDLLA-COOH were completely covered with globules after 7-day immersion. The amount of precipitation on the surface of PDLLA-COOH appeared to be greater than that observed on PDLLA-R. However, it is difficult to find evidence to account for the difference in the amount of precipitation from FE-SEM observation alone. Figure 5 shows TF-XRD patterns for the precipitates on the fibrous PDLLAs after immersion in HBS solution for 7 days. For the fibrous PDLLAs immersed for 7 days, two peaks assignable to apatite (JCPDS Card no. 09-0432) were observed at 25.86 and 31.76°. Moreover, the TF-XRD pattern of PDLLA-COOH showed relatively stronger apatite peak intensities compared with those observed for PDLLA-R. Generally, carboxyl (COOH) groups are known to induce apatite nucleation. Tanahashi and Matsuda [12] investigated the dependence of apatite formation in simulated body fluid on the surface functional group. The authors explained that biomineralization such as apatite formation predominantly occurs by calcium ion adsorption on the artificial material. Likewise, Sato et al. [13] investigated the apatite crystal nucleation mechanism on organic monolayers in a simulated body environment. The authors indicated that interfacial interaction between carboxyl groups and calcium ions was an important factor with respect to apatite formation in a simulated body environment, causing hydroxyapatite nucleation. On that basis, the present fibrous PDLLA-COOH is predicted to have a higher *in vivo* apatite formation ability than PDLLA-R. This finding within the limitation speculates that the electrospun fibrous PDLLA, having high specific surface area and porous structure, has more effectiveness of COOH to biological activity in comparison with bulk material. However, more detailed analysis of the fibrous PDLLA and its biological activity such as for cultured cell growth and its tissue response will be investigated further.

4. Conclusions

In this study, fibrous PDLLA was successfully prepared by electrospinning for use as a scaffold. The following is a summary of the authors' interpretation of the results.

(1) A 15 wt% PDLLA solution was the most beneficial for preparing a fibrous structure. In contrast, solutions of 5 or 10 wt% did not yield a completely fibrous structure.

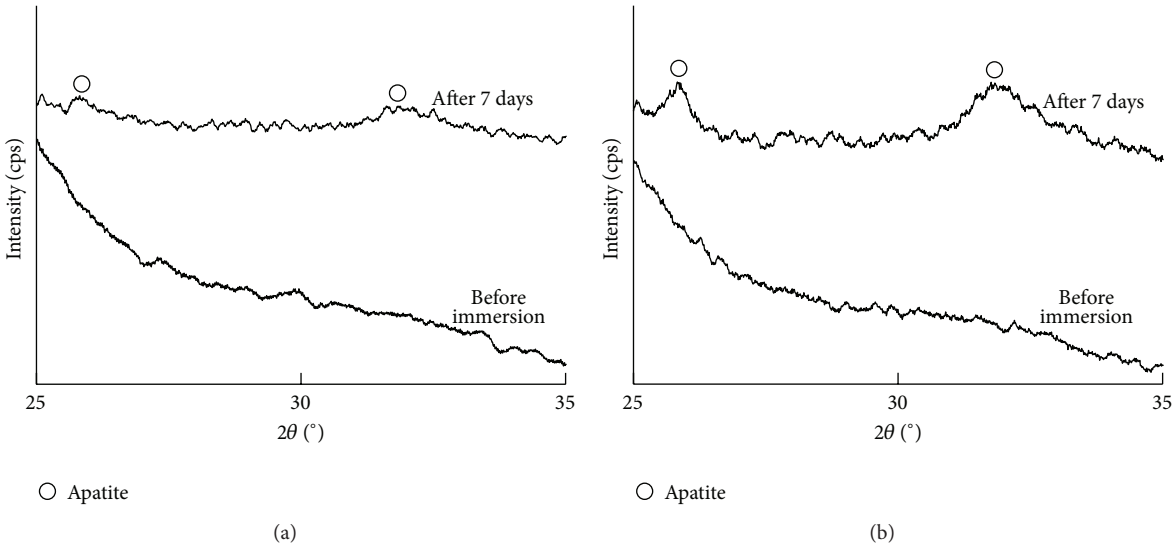

Figure 5: TF-XRD patterns of the PDLLA. Both PDLLA-R and PDLLA-COOH exhibit apatite peaks at 25.86° and 31.76°. (a) PDLLA-R; (b) PDLLA-COOH.

(2) PDLLAs prepared with various flow rates showed no differences in the morphology of the obtained fibrous structure.

(3) Apatite precipitate was formed on the PDLLA only 1 day after immersion in HBS solution. After 7 days of immersion, the apatite formation ability of the PDLLA-COOH was greater than that of the PDLLA-R, indicating that the carboxyl (COOH) group is effective for apatite activity in HBS solution.

Conflict of Interests

The authors declare that no competing interests exist.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Young Scientists (B) (no. 21791954) (24792155) from the Japan Society for the Promotion of Science.

References

[1] A. A. Ignatius, O. Betz, P. Augat, and L. E. Claes, "*In vivo* investigations on composites made of resorbable ceramics and poly(lactide) used as bone graft substitutes," *Journal of Biomedical Materials Research B*, vol. 58, no. 6, pp. 701–709, 2001.

[2] Q. Lu, A. Simionescu, and N. Vyavahare, "Novel capillary channel fiber scaffolds for guided tissue engineering," *Acta Biomaterialia*, vol. 1, no. 6, pp. 607–614, 2005.

[3] Y. Tanimoto and N. Nishiyama, "Preparation and in vitro behavior of a poly(lactic acid)-fiber/ hydroxyapatite composite sheet," *Advances in Materials Science and Engineering*, vol. 2009, Article ID 827241, 2009.

[4] S. I. Jeong, E. K. Ko, J. Yum, C. H. Jung, Y. M. Lee, and H. Shin, "Nanofibrous poly(lactic acid)/hydroxyapatite composite scaffolds for guided tissue regeneration," *Macromolecular Bioscience*, vol. 8, no. 4, pp. 328–338, 2008.

[5] D. Li, Y. Wang, and Y. Xia, "Electrospinning nanofibers as uniaxially aligned arrays and layer-by-layer stacked films," *Advanced Materials*, vol. 16, no. 4, pp. 361–366, 2004.

[6] M. M. Munir, F. Lskander, R. Khairur, and K. Okuyama, "High performance electrospinning system for fabricating highly uniform polymer nanofibers," *Review of Scientific Instruments*, vol. 80, Article ID 026106, 2009.

[7] W. Cui, X. Li, S. Zhou, and J. Weng, "In situ growth of hydroxyapatite within electrospun poly(DL-lactide) fibers," *Journal of Biomedical Materials Research A*, vol. 82, no. 4, pp. 831–841, 2007.

[8] N. T. Hiep and B. T. Lee, "Electro-spinning of PLGA/PCL blends for tissue engineering and their biocompatibility," *Journal of Materials Science: Materials in Medicine*, vol. 21, no. 6, pp. 1969–1978, 2010.

[9] M. Ngiam, S. Liao, A. J. Patil, Z. Cheng, C. K. Chan, and S. Ramakrishna, "The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering," *Bone*, vol. 45, no. 1, pp. 4–16, 2009.

[10] Y. Tanimoto, T. Hayakawa, T. Sakae, and K. Nemoto, "Characterization and bioactivity of tape-cast and sintered TCP sheets," *Journal of Biomedical Materials Research - Part A*, vol. 76, no. 3, pp. 571–579, 2006.

[11] T. Kokubo, M. Hanakawa, M. Kawashita et al., "Apatite formation on non-woven fabric of carboxymethylated chitin in SBF," *Biomaterials*, vol. 25, no. 18, pp. 4485–4488, 2004.

[12] M. Tanahashi and T. Matsuda, "Surface functional group dependence on apatite formation on self-assembled monolayers in a simulated body fluid," *Journal of Biomedical Materials Research*, vol. 34, no. 3, pp. 305–315, 1997.

[13] K. Sato, Y. Kumagai, and J. Tanaka, "Apatite formation on organic monolayers in simulated body environment," *Journal of Biomedical Materials Research*, vol. 50, no. 1, pp. 16–20, 2000.

[14] K. Kitahara, M. Fuse, and M. Fukumoto, "Effect of the introduction of a carboxylic acid group onto the surface of

a three-dimensional porous poly (lactic acid) scaffold on apatite deposition," *International Journal of Oral-Medical Sciences*, vol. 10, no. 3, pp. 179–186, 2011.

[15] T. Hanawa, M. Kon, H. Ukai, K. Murakami, Y. Miyamoto, and K. Asaoka, "Surface modification of titanium in calcium-ion-containing solutions," *Journal of Biomedical Materials Research*, vol. 34, no. 3, pp. 273–278, 1997.

[16] Y. Wan, X. Cao, S. Zhang, S. Wang, and Q. Wu, "Fibrous poly(chitosan-g-dl-lactic acid) scaffolds prepared via electro-wet-spinning," *Acta Biomaterialia*, vol. 4, no. 4, pp. 876–886, 2008.

[17] Z. M. Huang, Y. Z. Zhang, M. Kotaki, and S. Ramakrishna, "A review on polymer nanofibers by electrospinning and their applications in nanocomposites," *Composites Science and Technology*, vol. 63, no. 15, pp. 2223–2253, 2003.