Preparation and Characterization of Complex Gel of Type I Collagen and Aluminosilicate Containing Imogolite Nanofibers

Asuka Nakano,[1] Naozumi Teramoto,[1] Guoping Chen,[2] Yoshiko Miura,[3] Mitsuhiro Shibata[1]

[1]*Department of Life and Environmental Sciences, Faculty of Engineering, Chiba Institute of Technology, Narashino, Chiba 275-0016, Japan*
[2]*Biomaterials Center, National Institute for Materials Science, Tsukuba, Ibaraki 305-0044, Japan*
[3]*School of Materials Science, Japan Advanced Institute of Science and Technology, Nomi, Ishikawa 923-1292, Japan*

Received 24 December 2008; accepted 2 August 2009
DOI 10.1002/app.31199
Published online 22 June 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Complex gel materials of Type I collagen and aluminosilicate containing imogolite nanofibers were prepared as opaque gel by mixing an acidic fine dispersion of aluminosilicate with an acidic solution of collagen. The product was stained blue by Coomassie Brilliant Blue (CBB), indicating that the gel contained collagen. A white sponge was obtained after lyophilization of the complex gel. Elemental analysis revealed that the complex contains C, H, N, Al, and Si atoms; and the compositional ratio of aluminosilicate/collagen (w/w) was calculated as 0.75 for the complex gel when aluminosilicate was mixed with an equal quantity of collagen. Transmission electron microscope (TEM) observation showed that aluminosilicate nanofibers were homogeneously distributed in the collagen matrix. The thermogravimetric analysis (TGA) curve of the complex was not a simple summation of each components, and especially, the weight loss step corresponding to detachment of the adsorbed water observed in aluminosilicate became difficult to distinguish, suggesting that the adsorbed water was removed in the complexation. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 2284–2290, 2010

Key words: collagen; imogolite; nanostructures; fibers

INTRODUCTION

Interactions of biopolymers with inorganic nanostructured particles are very attractive to fabrication of nanocomposites for biofunctional materials by bottom-up technologies.[1] Well-known composite materials in living organisms are bone and scale, which are composed of collagen and hydroxyapatite crystals arranged in a certain direction.[2,3] Mineralized-collagen is an interesting material that can be produced by self-assembly at ambient temperatures.[4] Collagen is also found in extracellular matrix (ECM) and plays an important role as a mechanical reinforcing fibrous material in connective tissue such as tendon and cartilage. Therefore, collagen is used for biocompatible scaffold materials in a wide range. Various attempts have been made to fabricate collagen-based nanocomposites using nanostructured inorganic materials, such as hydroxyapatite,[4–6] tricalcium phosphate,[7] bioactive glass nanofibers,[8] layered silicate,[9–12] and carbon nanotubes.[13] The fabrication of nanofibers is reported to draw much advantage for cell cultivation. Ma and his coworkers have vigorously researched and suggest that the nanofibrous scaffolds promotes the adsorption of serum proteins and attachment of cells.[14–17] Zhang and his coworkers demonstrated that self-assembling peptide nanofiber scaffolds have broad applications including 3D-cell culture for regenerative medicine.[18–20] It is attractive to study the interaction of nanofibrous materials with biopolymers.

Imogolite is a hydrated-aluminosilicate polymer with tubular structure. The tubular structure is formed with an external diameter of ca. 2 nm, an internal diameter of ca. 1 nm, and a length from several hundred nanometers to several micrometers.[21] Imogolite is a naturally occurring material, commonly observed in the clay fraction of soils derived from glassy volcanic ashes. Since a synthetic pathway was established, imogolite is steadily available.[22,23] Although imogolite has an interesting nanostructure, there are only a few reports about its interaction with biopolymers.[24–26] Hirai et al. investigated the liquid crystal structure in the mixture of tunicate cellulose and imogolite in aqueous solution and prepared the nanocomposite film with high modulus.[24] Kondo and coworkers fabricated imogolite on the surface of the honeycomb-patterned

Correspondence to: N. Teramoto (teramoto-n@sea.it-chiba.ac.jp).

cellulose film.[25] Takahara and coworkers have energetically studied the preparation of polymer composites and hybrids containing imogolite.[27] Among a series of their studies, they prepared the hybrid gel of imogolite with pepsin whose activity was retained after four repeated reactions.[26] They also reported poly(vinyl alcohol) (PVA)/imogolite nanocomposites[28]; and mechanical and thermal properties of PVA were improved by addition of imogolite. We have a perspective that interactions between matrix and nanofibers are very important for improving the mechanical properties of matrix. Mechanical strength of collagen gel is very low under hydrated conditions, and the inorganic nanofibers, if it can disperse finely and interact with collagen, will be a most qualified candidate for a reinforcement filler.

In this study, we prepared composites of collagen with synthetic aluminosilicate containing imogolite nanofibers. Characterization and microscope observation of the composites were carried out, and the interaction between collagen and aluminosilicate was discussed. The potential application of this composite gel is in tissue engineering using biomaterial such as a scaffold for cell proliferation. We used Type I collagen from calf skin because of ready availability in this study. Extraction of collagen from domestic animals is reasonable in view of effective utilization of waste from food processing industry, though it has a potential risk of infectious diseases.

EXPERIMENTAL PART

Materials

Aluminum (III) chloride hexahydrate was purchased from Kanto Chemical Co. (Tokyo, Japan); and tetrasodium monosilicate *n*-hydrate (Si $\sim$ 22%) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Collagen, Type I, from calf skin (C9791) was purchased from Sigma-Aldrich Co. (MO) and used as received. The elemental content of the collagen was C 40.5%, H 6.2%, and N 7.4% (wt %) by elemental analysis. Ultra pure water (electric resistance $>$18 MΩ cm^{-1}) was obtained through Milipore Direct-Q and used for preparation of imogolite and the complex.

Synthesis of aluminosilicate containing imogolite

Imogolite was synthesized by the method of Suzuki et al.[23] as follows. To prepare the reactant solutions, 3.35 g of tetrasodium monosilicate *n*-hydrate (Si $\sim$ 22%) (0.012 mol) was dissolved in 200 mL of water; and 7.24 g of aluminum chloride hexahydrate (0.030 mol) was dissolved in 200 mL of water. To the solution of $AlCl_3$ was added $2Na_2O{\bullet}SiO_2$ solution dropwise and stirred for 20 min at room temperature. Then, 1*N* NaOH was added at a rate of 1 mL/min using a syringe pump under stirring until the pH of the mixture reached 7.0. The mixture was centrifuged at a rate of 3500 rpm for 10 min with polypropylene (PP) centrifuge tubes, and supernatant was removed to obtain aluminosilicate gel. For the purpose of washing out the excess salt, enough water was added to the PP centrifuge tubes to suspend the product and the suspension was poured into 400 mL of water in a 500 mL beaker. The suspension was stirred for 1 h at room temperature, and then centrifuged at a rate of 3500 rpm for 10 min, followed by removing the supernatant. This washing procedure was repeated three times. The precipitate, translucent gel, was suspended in 2 L of water, and pH of the suspension was adjusted to 4.0 with 0.5*N* HCl. After stirring for 2 days at room temperature, the suspension became almost transparent. The suspension was divided into two 1 L PP screw capped bottles and aged for 2 days in an electric oven set at 105°C. Then the suspension was lyophilized for 2 days to yield a white fibrous powder. The transmission electron microscope (TEM) observation revealed that nanofiber content was about 30%. The X-ray diffraction (XRD) analysis also confirmed the existence of imogolite nanofibers.

Preparation of complex of collagen with aluminosilicate

Collagen was dissolved in 0.05*N* acetic acid to obtain a 0.5 mg/mL solution. After a small amount of 1*N* NaOH ($\sim$ 1.7% volume of the collagen solution) was added for adjusting pH to 4, the resulting collagen solution was added dropwise to 1 mg/mL aluminosilicate suspension in 0.05*N* sodium acetate buffer (pH 4). The total volume of the combined solution was constant and the each volume of collagen solution and AS suspension is summarized in Table I. The combined solution was agitated for 24 h and centrifuged at a rate of 3500 rpm for 10 min. The precipitate, white opaque gel, was washed with

TABLE I
Volume of Collagen Solution and AS Suspension Fed in the Complex Preparation

Entry	Volume of 0.5 mg/mL collagen solution (mL)	Volume of 1 mg/mL AS suspension (mL)	Feed ratio (g-AS/ g-collagen)	Yield[a] (%)
1	48	12	0.5	27
2	40	20	1.0	20
3	30	30	2.0	37

[a] The yield was calculated from the weight of the lyophilized-complex gel and the total mass of AS and collagen fed in the experiments.

0.05*M* sodium acetate buffer (pH 4) and water, followed by lyophilization to yield a white cake.

Characterization

Elemental analysis for C, H, and N was performed on a Sumigraph NCH-21 elemental analyzer with a thermal conductive detector (TCD) (Sumika Chemical Analysis Service, Ltd., Tokyo, Japan). Elemental analysis for Al and Si was performed on an IRIS Advantage inductively coupled plasma atomic emission spectrometer (ICP-AES) (Thermo Jarrell Ash Co., MA). Samples were pretreated as follows: the test sample was carbonized by sulfuric acid, followed by ashing; then, the resulting ash was solubilized by fusion with the alkaline agent. The surface morphology was observed by a Hitachi S-4700 field emission scanning electron microscope (FE-SEM); the accelerating voltage was 1 kV or 5 kV; and the samples were coated with gold before the observation. The nano-order morphology of aluminosilicate and composites was observed by a Hitachi HF-2000 field emission transmission electron microscope (FE-TEM). Each sample was suspended in water, and the suspension was cast on a grid followed by drying for 24 h at room temperature. The accelerating voltage was 200 kV.

UV–Vis spectra of solutions were recorded on a Jasco V-650 UV–Vis spectrophotometer using a quartz cell of 1-cm path length. Thermogravimetric analysis (TGA) was conducted with a Perkin Elmer thermogravimetric analyzer TGA 7 at a heating rate of 20°C/min under a nitrogen atmosphere. The weight loss curve was plotted against temperature for further analysis. Infra-red (IR) spectra were recorded on a Shimadzu FT-IR 8400S spectrometer by the attenuated total reflection (ATR) method for collagen and the potassium bromide (KBr) method for composites.

RESULTS AND DISCUSSION

Characterization of the complex of collagen with aluminosilicate

Aluminosilicate containing imogolite (AS) was dispersed very well in a sodium acetate buffer (pH 4.0) to yield a transparent solution at a concentration of 1 mg/mL. Because collagen we used was not dissolved completely in the same buffer, collagen was dissolved in 0.05*N* acetic acid and then small amount of sodium hydroxide was added to adjust pH of the solution to 4. When these solutions were combined, the mixture got turbid whitely and then white flocculus precipitated to yield opaque gel by centrifugation. The presence of collagen in the flocculus was confirmed by staining with Coomassie Brilliant Blue (CBB) G-250 (Nacalai Tesque, Kyoto, Japan) solution. The CBB solution was added to the AS/collagen complex gel and the dispersion was mixed well at room temperature. The complex gel dispersion turned blue (Fig. 1, right). For comparison, AS gel was prepared as a blank without protein by adding ammonia water to AS acidic dispersion. When the CBB solution was added to the AS gel dispersion, almost no change in color was observed (Fig. 1, left). The result strongly suggests that the former gel prepared from AS and collagen contains protein. We tried to carry out the quantitative analysis by CBB and UV–Vis spectroscopy using supernatant of the combined solution. However, we could not determine the amount of collagen in the supernatant, because the acidity of the solution inhibited the quantitative detection of protein with CBB and AS aggregated particles remaining in the solution scattered light especially with shorter wavelength.

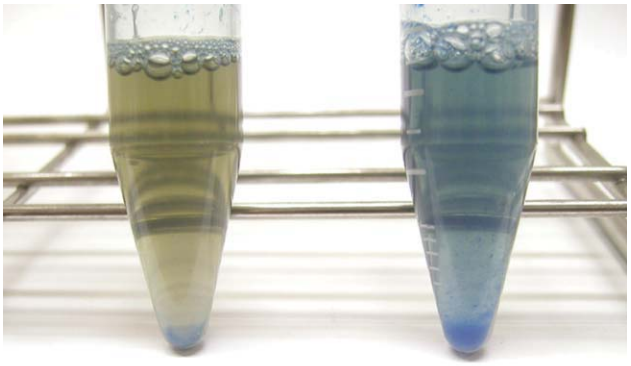

Figure 1 Digital photographs of the gel dispersion in CBB staining solution: (left) AS gel and (right) AS/collagen complex gel.

When the feed ratio of AS to collagen (AS/collagen) was changed from 1.0 to 0.5 and 2.0, the white flocculus precipitation appeared in all cases. To assess the composition of the precipitate, elemental analysis of the samples was carried out after washing several times with the buffer and water and subsequent lyophilization. The result is summarized in Table II. The compositional ratios of the precipitates were calculated from the Si/N ratios using the value of Si content of AS/N content of collagen (1.10 g/g) which is determined by the discrete elemental analyses of AS and collagen. When the AS/collagen feed ratio decreased from 1.0 to 0.5, the compositional ratio did not change; the AS/collagen compositional ratio was ca. 0.7. The result implies that the excess collagen did not precipitate with AS under the present experimental condition. In the case where the feed ratio AS/collagen is 0.5, collagen covered AS surface and the bundle was ionically stabilized in the solution, aggregated, and precipitated;

TABLE II
Compositional Ratio of AS/Collagen Complex

Entry	Feed ratio (g-AS/g-collagen)	Si/N ratio (g/g)	Si/C ratio (g/g)	Compositional ratio[a] (g-AS/g-collagen)
1	0.5	0.80	0.22	0.73
2	1.0	0.82	0.25	0.75
3	2.0	1.34	0.32	1.22

[a] The compositional ratio was calculated from the Si/N ratio.

and the excess collagen remaining in the supernatant did not precipitate. In the case where the As/collagen feed ratio was 1.0, only a part of AS whose surface was covered with sufficient collagen precipitated and the residual part of AS remained dispersed in the buffer. On the other hand, when the AS/collagen feed ratio increased to 2.0, the AS/collagen compositional ratio increased to 1.2. In this case, AS dominated in the bundle where AS was entangled with collagen. However, the compositional ratio AS/collagen did not reach 2.0, implying that excess AS remaining in the supernatant also did not precipitate with collagen. Yamamoto et al. reported the dispersion of conjugated anionic polymer hybrid with imogolite.[29] They also considered the bundle structure in which the anionic polymer covered imogolite.

The morphology of the lyophilized complex gel (Entry 2, obtained at the AS/collagen feed ratio of 1.0) was observed by SEM, and the photographs are shown in Figure 2. The lyophilized gel does not have fibrous morphology but macro porous morphology (in the low magnification) with micro wrinkles on the wall (in the high magnification). The wrinkles may represent the presence of the aggregation of fibrous collagen and fibrous AS, i.e., imogolite. TEM photographs of AS and the complex (Entry 2) are shown in Figure 3. The photographs show the nanofiber regions of each material. The magnifying view of the complex revealed that imogolite nanofibers were homogeneously surrounded by collagen matrix.

Interaction between collagen and aluminosilicate

To assess the interaction between collagen and AS, first, the gel formation was carried out in the presence of 5*M* urea. Urea is known to interfere with the hydrogen bond formation. When collagen aqueous solution was added to AS suspension in the presence of 5*M* urea, white precipitate was sedimented (24% yield). This precipitate is confirmed to contain collagen and AS by measuring the FTIR spectrum. The result suggests that the major interaction between collagen and AS was not the hydrogen bond. Then, the lyophilized-AS/collagen complex obtained without urea was immersed in phosphate buffer solutions whose pH varied from 4 to 10 to investigate whether the interaction between collagen and AS is the electrostatic interaction. The resulting supernatants were analyzed by UV–Vis spectrophotometer (Fig. 4). If the binding force is due to the electrostatic interaction, collagen will be eluted from the complex gel as pH changes and its absorbance will be detected by UV–Vis spectroscopy. The absorbance at 270 nm increased remarkably as pH increased to 10, whereas the absorbance was not significant at pH 4 to 8. AS nanofibers, imogolite, is positively charged at the pH below 6,[22] and it can interact with negatively charged

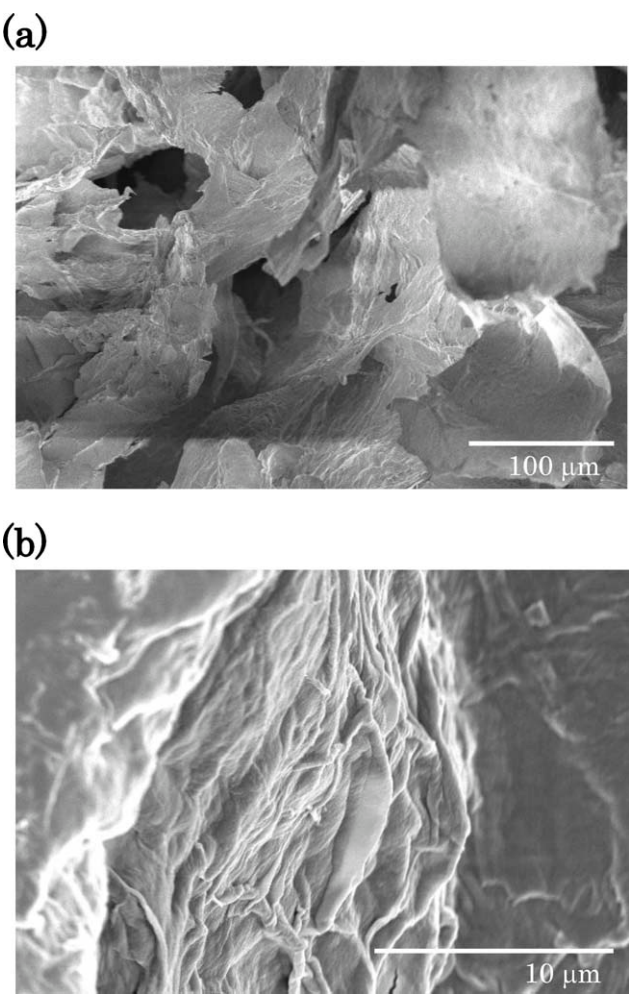

Figure 2 SEM photographs of AS/collagen complex gel: (a) low and (b) high magnification.

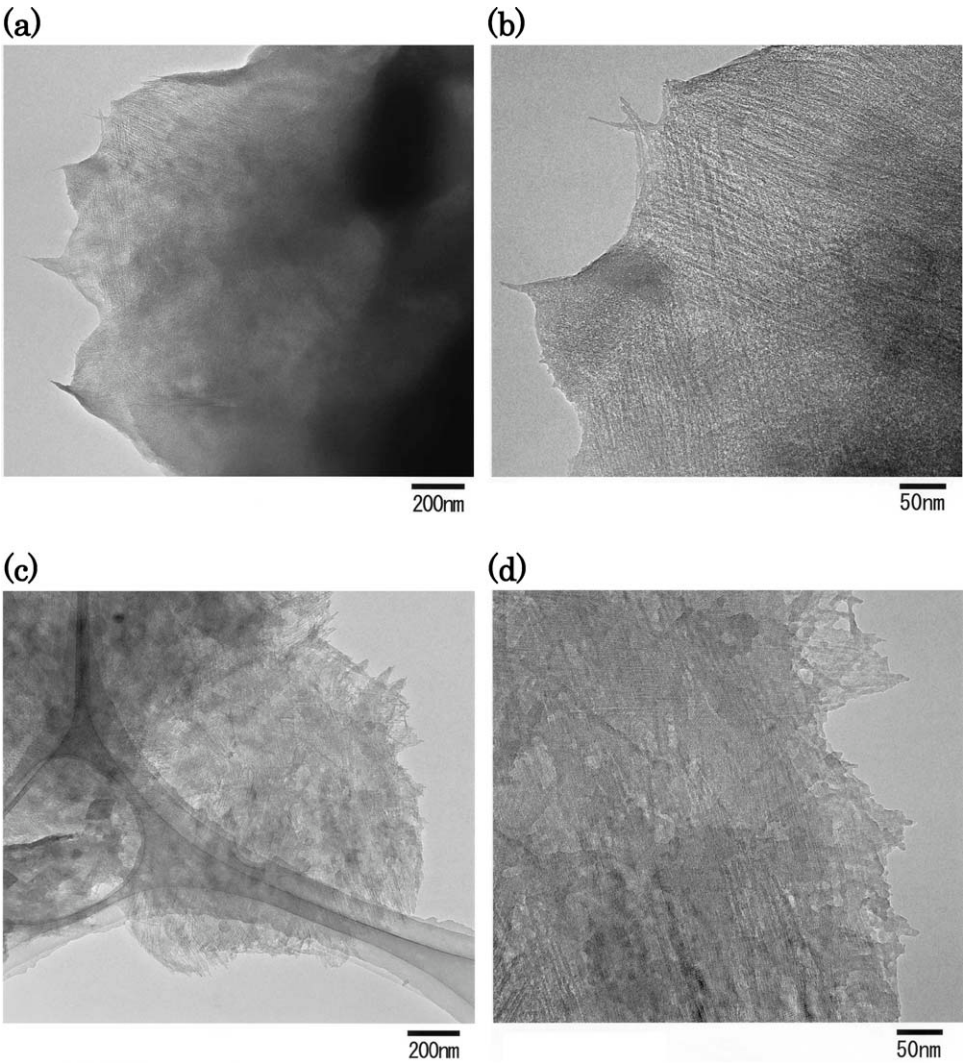

Figure 3 TEM photographs of (a, b) AS and (c, d) AS/collagen complex gel: (a, c) low and (b, d) high magnification.

molecules. Inoue et al.[26] reported that imogolite interacted with pepsine at pH 3.1; because pepsin, whose isoelectric point is 1.0, was negatively charged. Considering that the isoelectric point of collagen is between 7 to 8,[30,31] collagen should be positively charged at pH 4 in our condition for preparing the complex. Therefore, we could not conclude that the interaction between AS and collagen was exclusively electrostatic interaction. However, according to the dissociation behavior especially at higher pH, electrostatic interaction may be dominant. When we tried combining AS suspension with the solution of poly(L-lysine) which has no carboxylic group, no precipitation occurred at pH 4. We have an assumption that, in a collagen molecule, there exist multiple points which have localized negative charges and can interact with AS. Indeed, collagen contains many acidic amino acid residues, such as glutamic acid and aspartic acid, which can be negatively charged at pH 4. The example of the interaction of collagen and chitosan, a cationic polymer, was reported by Taravel and Domard,[32,33] and Sionkowska et al.[34] These reports support our assumption.

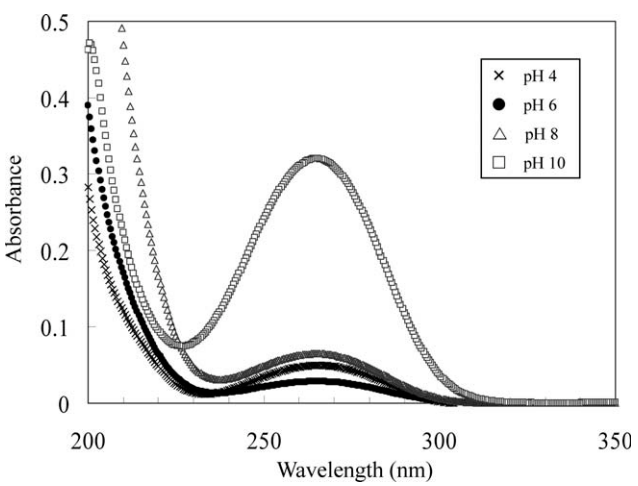

Figure 4 UV spectra of supernatant of AS/collagen complex gel immersed in buffer solutions at various pHs.

Figure 5 shows the IR spectra of lyophilized-collagen and AS/collagen complex. In these spectra, two major absorption bands were observed: one is assigned to the amide I at 1650 cm^{-1} corresponding to the stretching vibration of C=O, and the other is assigned to the amide II at 1550 cm^{-1} corresponding to the bending vibration of N—H. According to the literature,[35] the absorption band of the O—H bending vibration of water appears at around 1640 cm^{-1}, which causes the apparent increase of the amide I absorption. The amide I absorption of AS/collagen complex relative to the amide II is smaller than that of collagen, implying that some water molecules attached to collagen was removed in the preparation of AS/collagen complex. The absorption band at around 900–1000 cm^{-1} observed in the spectrum of AS/collagen complex is attributed to the stretching vibration of Al—O—Si of AS. The peak at 1740 cm^{-1} in the spectrum of collagen is unknown, which may be attributed to the ester C=O stretching vibration of contaminated lipid, sometimes seen in the collagen sample.[36] The small peak at 1710 cm^{-1} is attributed to the nonionized carboxyl C=O stretching vibration of side chains.[37] This peak disappeared in the spectrum of AS/collagen complex and a new peak appeared at 1570 cm^{-1} and 1400 cm^{-1} attributed to the ionized carboxyl groups (COO— asymmetry stretching and symmetry stretching, respectively).[35,38,39] These spectral changes support the possibility of the electrostatic interaction between collagen and AS.

The weight loss profile of the lyophilized-AS/collagen complex at rising temperature was measured by TGA and shown in Figure 6. The TGA curves of collagen and AS are also shown for comparison. Collagen exhibited a two-step degradation profile: the first step (150–200°C) corresponds to the elimination

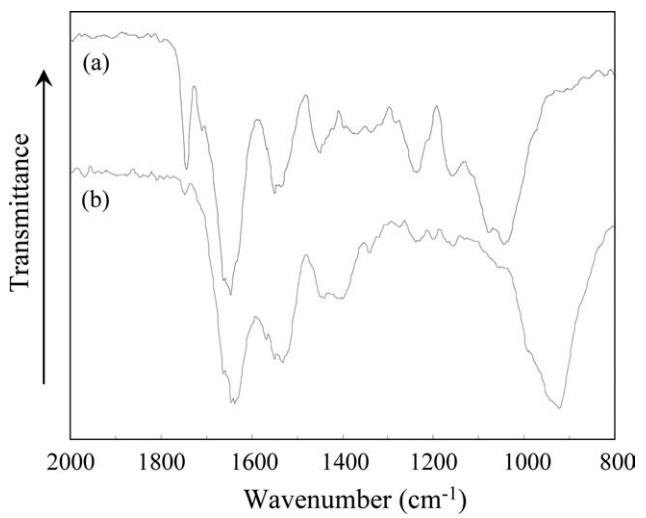

Figure 5 FTIR spectra of (a) collagen and (b) AS/collagen.

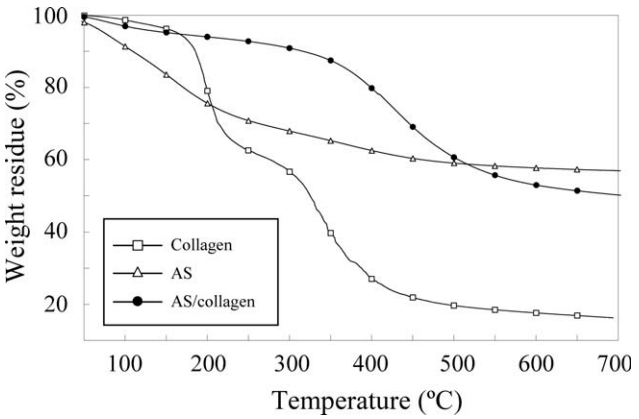

Figure 6 TGA curves of collagen, AS, and AS/collagen complex gel.

reaction such as dehydration and decarboxylation at the side chains of collagen: the second step (300–400°C) corresponds to the degradation of the main chain. In the TGA curve of AS, any remarkable degradation step was not found; and the weight gradually decreased during heating until the temperature reached ~ 400°C. The gradual weight decrease corresponds to the loss of the adsorbed water (100–200°C) and the structured water (300–400°C) on the AS.[23] Interestingly, the TGA curve of AS/collagen complex was not the averaged curve of the summation of the collagen profile and the AS profile. The steps corresponding to the first degradation of collagen and the loss of the adsorbed water on AS were not found. Therefore, the rate of the weight decrease was very slow until ~ 300°C. The degradation was accelerated at 300–400°C and the AS/collagen complex exhibited one-step degradation profile. This step may correspond to the degradation of collagen. Disappearing of weight loss steps which indicate the detachment of water from AS surface and collagen side chains reflects the close interaction between the collagen side chains and AS surface. It is reasonable to consider that the binding site for water molecules on AS was occupied by collagen side chains. The result suggests that the AS/collagen composite was not the phase-separated mixture, but the hybrid in which collagen is closely bound to AS.

The mechanical properties of AS/collagen composite gel are now under investigation and the results will appear in the near future.

CONCLUSIONS

1. Complex gel of Type I collagen and aluminosilicate (AS) containing imogolite nanofibers was prepared by addition of the collagen solution to the AS fine dispersion at pH 4. The complex gel was composed of collagen and AS. When feed

ratio of AS to collagen (AS/collagen) varied from 0.5 to 2.0; the compositional ratio of AS/collagen in complex gel was varied from 0.7 to 1.2. SEM showed that morphology of the lyophilized-gel was not fibrous but macro porous, and there were many micro wrinkles on the wall. TEM revealed that imogolite nanofibers were homogeneously surrounded by collgen matrix.

2. The collagen was eluted from complex gel in buffer solutions at pH 10, but not significantly at pH 4 to 8. The result implies that the electrostatic interaction is mainly involved in the complex formation of collagen with AS. IR spectral change of collagen suggests that the nonionized carboxyl groups were changed to the ionized carboxyl groups in the presence of AS. The result supports the possibility of the electrostatic interaction between collagen and AS. The TGA curves of AS/collagen complex was not the averaged curves of the summation of the collagen TGA profile and the AS TGA profile. This result suggests that collagen was closely bound to AS in the complex gel by substituting for absorbed water on AS.

The authors greatly acknowledge Dr. Masaya Suzuki, National Institute of Advanced Industrial Science and Technology (AIST), Japan, for providing a synthetic imogolite sample and giving advices for the synthetic procedures.

References

1. Ruiz-Hitzky, E.; Darder, M.; Aranda, P. J Mater Chem 2005, 15, 3650.
2. Cui, F. Z.; Li, Y.; Ge, J. Mater Sci Eng R Rep 2007, 57, 1.
3. Onozato, H.; Watabe, N. Cell Tissue Res 1979, 201, 409.
4. Kikuchi, M.; Ikoma, T.; Itoh, S.; Matsumoto, H. N.; Koyama, Y.; Takakuda, K.; Shinomiya, K.; Tanaka, J. Compos Sci Technol 2004, 64, 819.
5. Du, C.; Cui, F. Z.; Zhu, X. D.; De Groot, K. J Biomed Mater Res 1999, 44, 407.
6. Lin, X.; Li, X.; Fan, H.; Wen, X.; Lu, J.; Zhang, X. Mater Lett 2004, 58, 3569.
7. Shibata, Y.; Yamamoto, H.; Miyazaki, T. J Dent Res 2005, 84, 827.
8. Kim, H. W.; Song, J. H.; Kim, H. E. J Biomed Mater Res A 2006, 79, 698.
9. Darder, M.; Ruiz, A. I.; Aranda, P.; Van Damme, H.; Ruiz-Hitzky, E. Curr Nanosci 2006, 2, 231.
10. Sun, Y.; Zhou, Y.; Ye, X.; Chen, J.; Wang, Z. Mater Lett 2008, 62, 2943.
11. Zhuang, H.; Zheng, J. P.; Gao, H.; Yao, K. D. J Mater Sci Mater Med 2007, 18, 951.
12. Teramoto, N.; Uchiumi, D.; Niikura, A.; Someya, Y.; Shibata, M. J Appl Polym Sci 2007, 106, 4024.
13. Macdonald, R. A.; Laurenzi, B. F.; Viswanathan, G.; Ajayan, P. M.; Stegemann, J. P. J Biomed Mater Res A 2005, 74, 489.
14. Ma, P. X. Adv Drug Deliv Rev 2008, 60, 184.
15. Woo, K. M.; Chen, V. J.; Ma, P. X. J Biomed Mater Res A 2003, 67, 531.
16. Chen, V. J.; Smith, L. A.; Ma, P. X. Biomaterials 2006, 27, 3973.
17. Woo, K. M.; Jun, J.-H.; Chen, V. J.; Seo, J.; Baek, J.-H.; Ryoo, H.-M.; Kim, G.-S.; Somerman, M. J.; Ma, P. X. Biomaterials 2007, 28, 335.
18. Yang, Y.; Khoe, U.; Wang, X.; Horii, A.; Yokoi, H.; Zhang, S. Nano Today 2009, 4, 193.
19. Holmes, T. C.; De Lacalle, S.; Su, X.; Liu, G.; Rich, A.; Zhang, S. Proc Natl Acad Sci USA 2000, 97, 6728.
20. Ellis-Behnke, R. G.; Liang, Y.-X.; You, S.-W.; Tay, D. K. C.; Zhang, S.; So, K.-F.; Schneider, G. E. Proc Natl Acad Sci USA 2006, 103, 5054.
21. Cradwick, P. D. G.; Farmer, V. C.; Russell, J. D.; Masson, C. R.; Wada, K.; Yoshinaga, N. Nat Phys Sci 1972, 240, 187.
22. Farmer, V. C.; Adams, M. J.; Fraser, A. R.; Palmieri, F. Clay Miner 1983, 18, 459.
23. Suzuki, M.; Inukai, K.; Maeda, M. J Vac Soc Jpn 2006, 49, 29.
24. Hirai, A.; Ikuno, M.; Horii, F.; Donkai, N.; Tsuji, M. Polym Prep Jpn 2005, 54, 720.
25. Suwa, T.; Kasai, W.; Kondo, T.; Morita, M. Polym Prep Jpn 2005, 54, 1148.
26. Inoue, N.; Otsuka, H.; Wada, S.; Takahara, A. Chem Lett 2006, 35, 194.
27. Yamamoto, K.; Otsuka, H.; Takahara, A. Polym J 2007, 39, 1.
28. Yamamoto, K.; Otsuka, H.; Wada, S.-I.; Sohn, D.; Takahara, A. Soft Matter 2005, 1, 372.
29. Yamamoto, K.; Jiravanivhanun, N.; Irie, A.; Otsuka, H.; Takahara, A. Polym Prep Jpn 2007, 56, 3017.
30. Highberger, J. H. J Am Chem Soc 1939, 61, 2302.
31. Zhang, Z.; Li, G.; Shi, B. J Soc Leather Technol Chem 2006, 90, 23.
32. Taravel, M. N.; Domard, A. Biomaterials 1993, 14, 930.
33. Taravel, M. N.; Domard, A. Biomaterials 1995, 16, 865.
34. Sionkowska, A.; Wisniewski, M.; Skopinska, J.; Kennedy, C. J.; Wess, T. J. Biomaterials 2004, 25, 795.
35. Jackson, M.; Choo, L.-P.; Watson, P. H.; Halliday, W. C.; Mantsch, H. H. Biochim Biophys Acta 1995, 1270, 1.
36. Küpper, L.; Heise, H. M.; Bechara, F. G.; Stücker, M. J Mol Struct 2001, 565, 497.
37. Degirmenbasi, N.; Kalyon, D. M.; Birinci, E. Coll Surf B 2006, 48, 42.
38. Park, S.-N.; Park, J.-C.; Kim, H. O.; Song, M. J.; Suh, H. Biomaterials 2002, 23, 1205.
39. Iconomidou, V. A.; Georgaka, M. E.; Chryssikos, G. D.; Gionis, V.; Megalofonou, P.; Hamodrakas, S. J. Int J Biol Macromol 2007, 41, 102.