J Mater Sci: Mater Med (2008) 19:2453–2461
DOI 10.1007/s10856-008-3370-0

Bioactive nanocomposite coatings of collagen/hydroxyapatite on titanium substrates

Shu-Hua Teng · Eun-Jung Lee · Chee-Sung Park · Won-Young Choi · Du-Sik Shin · Hyoun-Ee Kim

Received: 26 September 2007 / Accepted: 2 January 2008 / Published online: 25 January 2008

Abstract Collagen/hydroxyapatite (HA) nanocomposite thin films containing 10, 20, and 30 wt.% HA were prepared on commercially pure titanium substrates by the spin coating of their homogeneous sols. All of the nanocomposite coatings having a thickness of ~7.5 μm exhibited a uniform and dense surface, without any obvious aggregation of the HA particles. A minimum contact angle of 36.5° was obtained at 20 wt.% HA, suggesting that these coatings would exhibit the best hydrophilicity. The in vitro cellular assays revealed that the coating treatment of the Ti substrates favored the adhesion of osteoblast-like cells and significantly enhanced the cell proliferation rate. The cells on the nanocomposite coatings expressed much higher alkaline phosphatase (ALP) levels than those on the uncoated Ti substrates. Increasing the amount of HA resulted in a gradual improvement in the ALP activity. The nanocomposite coatings on Ti substrates also exhibited much better cell proliferation behaviors and osteogenic potentials than the conventional composite coatings with equivalent compositions, demonstrating the greater potential of the former as implant materials for hard tissue engineering.

1 Introduction

Titanium (Ti) and Ti alloys have been extensively explored for various biomedical applications, especially as dental and orthopaedic implants, due to their good mechanical properties, high corrosion resistance, and excellent biocompatibility [1, 2]. Nowadays, the surface modification of these materials is often performed to improve the bone-implant contact and acquire the desired biological properties, while retaining their advantageous bulk properties. Among the different materials and methods used for the surface modification of Ti substrates, coating them with hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$], the main inorganic component of human hard tissues, has attracted a great deal of attention over the last few decades. HA coatings showed high bioaffinity to cells and excellent osteoconductivity, which were expected to improve the biocompatibility, enhance the mechanical strength of bone bonding to Ti implants, and increase the amount of bone growth around the implants [3–5]. On the other hand, several potential drawbacks also exist in terms of the preparation and applications of HA coatings. Their inherently brittle behavior may lead to the formation of cracks in the coating layer during their preparation or heat-treatment [6]. Moreover, the relatively poor bonding strength of HA coatings on Ti substrates is likely to cause the premature failure of the bone–implant interface and the consequent dislodgment of the implants in vivo [7, 8]. The combination of the HA coatings with some bioresorbable polymers to form biocomposites has been considered as an efficient approach to tackle these issues [9, 10].

Type I collagen, the major structural protein of hard tissues, is a strong candidate for the surface modification of various substrates. Recent studies concerning collagen coatings on Ti hard-tissue implants have demonstrated their effective roles in stimulating cellular responses [11], increasing bone remodeling [12, 13], and improving bone growth and bone-implant contact [14]. Therefore, hybrids of collagen and HA have greater potential for clinical

S.-H. Teng · E.-J. Lee · C.-S. Park · W.-Y. Choi · D.-S. Shin · H.-E. Kim (✉)
Department of Materials Science and Engineering,
Seoul National University, Seoul 151-744, Republic of Korea
e-mail: kimhe@snu.ac.kr

applications than their pure HA or collagen equivalents, because they benefit from the advantageous properties of both materials, while retaining a similar composition and structure to that of human hard tissues. In the composite coatings, the collagen component can act as a matrix to embed the HA particles, thereby alleviating the brittleness of the HA coating and promoting the adhesion of the HA particles to the Ti substrate. At the same time, the HA particles used as a filler of the collagen matrix are expected to improve the mechanical strength and biological performance of the collagen coatings.

Several studies have been carried out on collagen/HA coatings, but only a limited number of methods of preparing them have been proposed so far, with biomimetic synthesis being the generally preferred technique [15–17]. The classical biomimetic process usually involves the immersion of the substrates in a collagen-contained simulated body fluid (SBF) or 1.5 times SBF (1.5SBF) for about 1 week to allow the co-precipitation of bone-like apatite crystals and collagen. Although the use of supersaturated SBF (5SBF) currently allows the collagen/HA coatings to be prepared within 1 day [18, 19], the control of the composition, chemical homogeneity, and thickness of the coatings remains a synthetic challenge.

In the present work, an integrated technique consisting of a modified mixing process and the spin coating method was explored to prepare uniform collagen/HA composite coatings on Ti substrates. The effects of the composition of the coatings on their morphology and surface properties were investigated by SEM and contact angle measurements, respectively. In addition, their biological properties were assessed by means of in vitro osteoblast-like cell culture tests.

2 Materials and methods

2.1 Materials

Acid-soluble Type I collagen (atelocollagen from calf skin, MW 300,000) was purchased from a commercial vendor (Elastin Products Company, Inc., USA). All other reagents used in the present study were of analytical grade and supplied by a vendor (Sigma–Aldrich Co. Ltd., USA), unless otherwise specified. Doubly distilled and deionized water was used throughout.

2.2 Preparation of nano-HA particles

Single phase HA powder was synthesized by a biomimetic method. Commercial $Ca(OH)_2$ and H_3PO_4 were chosen as the calcium and phosphate sources, respectively, for HA. The $Ca(OH)_2$ and H_3PO_4 solutions in stoichiometric proportions ([Ca]/[P] = 1.67) were simultaneously and separately added to a reaction vessel containing Tris–HCl buffer solution at 37°C and pH 9. After the titration, the resulting precipitate was aged at 37°C for 24 h under stirring, followed by centrifuging for 10 min at 2,500 rpm and washing with distilled water. Then, the precipitate was re-dispersed in water to make a slurry with a certain concentration. The total amount of HA in the slurry was considered to be approximately equal to the theoretical value, which was calculated on the basis of the assumption that Ca and P completely reacted to form HA. The slurry containing a required amount of HA was separated and further centrifuged at 14,000 rpm for 10 min to remove as much as possible of the water.

2.3 Coating of collagen/HA on Ti by spin coating

For the precursor of the spin coating, type I collagen sponge was dissolved at 7 w/v% in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), and then mixed with the prepared HA precipitates. After stirring at room temperature for 24 h, a collagen/HA nanocomposite was obtained in the form of a homogeneous, viscous sol. As substrates for the film deposition, commercially pure Ti discs (grade 2) with dimensions of $10 \times 10 \times 1.5\ mm^3$ were polished with #600 diamond pastes and cleaned ultrasonically in acetone, ethanol and distilled water successively for 20 min each. The spin coating procedure of the collagen/HA sols was performed in a WS-400-6NPP-LITE spinner (Laurell Technologies, North Wales, PA) at 3,000 rpm for 20 s, followed by drying the coated Ti substrates in a desiccator under vacuum overnight. Subsequently, the coatings were chemically cross-linked in 90% ethanol containing *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) hydrochloride and *N*-hydroxysuccinimide (NHS) solutions for 24 h at 4°C. After the cross-linking treatment, the coatings were washed with sufficient distilled water and freeze-dried. The obtained specimens were kept in the desiccator until use.

The chemical compositions of the coatings were controlled by adjusting the mixture used for their preparation. Composite coatings with 10, 20, and 30 wt.% HA were prepared and the corresponding specimens were designated as COL-10%HA, COL-20%HA, and COL-30%HA, respectively. A pure collagen coating was used as a control in this study. In addition, for the purpose of comparison, a conventional composite sol containing 20 wt.% HA was also prepared by mixing the collagen/HFP solution with commercial HA powder (Alfa Aesar Co., Milwaukee, WI, USA), and spin-coated on Ti substrates under the same conditions.

2.4 Characterization

A small portion of the precipitated HA powder was freeze-dried, and its phase was investigated by X-ray diffraction (XRD; M18XHF-SRA, Mac Science Co.) analysis at a scan rate of 0.5° min^{-1} with Cu Kα radiation ($\lambda = 1.54056$ nm). The HA slurry obtained after the last washing was re-suspended and one drop of it, placed on a copper grid coated with amorphous carbon film, was used to examine the morphology of the HA crystals by transmission electron microscopy (TEM; JEM-2000EXII, JEOL) and obtain their selected area diffraction (SAD) pattern. The surface and cross-sectional morphologies of the coating layers were observed with field emission scanning electron microscopy (FESEM; JSM-6330F, JEOL). The contact angles of the coatings were measured at room temperature by the sessile drop method with distilled water using a Phoenix 300 contact angle analyzer (Surface Electro Optics Corporation, Korea). At least 5 independent measurements were performed for each sample.

2.5 In vitro cellular assays

For the assessment of the cellular behaviors on the coating system in terms of the cell attachment, proliferation, and differentiation, the MC3T3-E1 cell line was used and pre-incubated in a culture medium consisting of α-modified minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 50 IU ml^{-1} of penicillin, and 50 μg ml^{-1} of streptomycin. The culture was maintained at 37°C in a humidified incubator with 5% CO_2, and the medium was changed every 3 days. Before the cell culture test, the disc specimens were placed on tissue-culture polystyrene (TCPS) plates and sterilized under ultraviolet (UV) light for 30 min.

For the attachment study, 1 ml of the MC3T3-E1 cell suspension having a density of 5×10^4 cells/ml was seeded on each scaffold and allowed to culture in the incubator for 1 day. Then, the cell-growth morphology was observed with SEM, after fixing, dehydrating, and gold-coating the cells.

To evaluate the biocompatibility of the coatings, the cell viability on the specimens was measured using a cell proliferation assay kit (Cell Titer 96® Aqueous One Solution, USA). Briefly, 1 ml of a cell suspension having a cell density of 3×10^4 cells/ml was plated on the UV-sterilized specimens. After harvesting the cells at 3 days, the culture medium was removed, 100 μl of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-caebozymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] solution in 1 ml of culture media added to each well, and the solutions left to stand at 37°C for 3 h. Finally, the colorimetric measurement of the specimens was performed using 200 μl aliquots of each solution on a spectrophotometer at 490 nm.

Three milliliters of the MC3T3-E1 cell suspension (1.5×10^4 cells/ml) was pipetted onto the coated Ti discs (25 mm in diameter and 3 mm in height), and the cells were cultured on the coatings for 14 days to examine the production of alkaline phosphatase (ALP), which is an important marker of osteogenic differentiation. The specimens were rinsed with PBS after the removal of the culture medium, and the cell layers were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) solution (Sigma, USA). After centrifugation, the cell pellets were re-suspended by treating them with 1% Triton X-100 (Sigma, USA) and a sonication process. The ALP activity was finally examined using *p*-nitrophenyl phosphate as the substrate to react with aliquots of each solution, following the manufacturer's protocol (Sigma ALP kit 104). The colorimetric measurement of the specimens was performed on a spectrophotometer at 405 nm, and the absorbance was converted to the ALP level based on a protein standard curve.

The cell proliferation and differentiation tests were performed on three replicate samples and the data were expressed as the mean ± standard deviation (SD). The Student's *t*-test was used to determine whether any statistically significant difference existed between the experimental groups. A difference between the groups was considered to be significant at $P < 0.05$ and $P < 0.01$.

3 Results and discussion

3.1 Coating process

Spin coating is a simple and effective technique for controlling the thickness of thin coatings [20, 21]. However, only a few attempts have been made to fabricate inorganic–organic composite films using this method, probably due to the difficulty involved in the preparation of a homogeneous precursor. To solve this problem, in this study, we proposed a modified procedure for preparing a uniform collagen/HA composite sol. The detailed experimental scheme is described in Fig. 1.

Ultrafine HA particles were first prepared by the simultaneous titration of $Ca(OH)_2$ and H_3PO_4 solutions. After the repeated centrifugation/washing process, the HA precipitates so obtained were further centrifuged at high speed to remove as much of the water as possible. As observed in our experiment, the presence of too much water in the HA precipitates tends to reduce the viscosity of the resultant collagen/HA composite sols and lead to the precipitation of collagen fibers from the HFP solution.

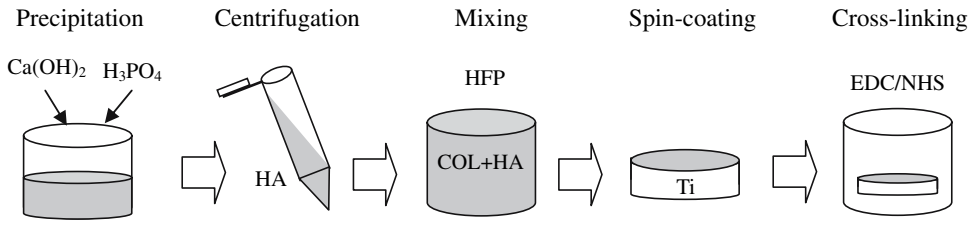

Fig. 1 Schematic illustration of the processes used to produce the collagen/HA composite coating on the Ti substrates

Subsequently, the gel-like HA precipitates, without any dry treatment, were directly re-dispersed in the collagen/HFP solution. A viscous, homogeneous composite sol was obtained by stirring the mixture for 24 h at room temperature, and it remained well blended for several days without any segregation occurring. This sol state of the nanocomposite was of particular importance in terms of the ability of the spin coating process to produce a uniform film on the Ti substrates. In contrast, the composite slurry prepared by the conventional mixing method only remained in its initial emulsion state for a few hours.

In order to obtain a suitable precursor for the spin coating, the HA content in the composite slurry was required to be less than 30 wt.% in this study. Increasing the HA content to more than 50 wt.% caused severe gelation of the composite precursor, thereby reducing its viscosity and fluidity. After the spin coating, the thin film that was obtained was cross-linked in EDC/NHS solution to ensure its chemical and structural stability, as presented in Fig. 1.

3.2 Phase and morphology of HA

The XRD pattern of the freeze-dried HA powder is depicted in Fig. 2. All of the diffraction peaks were indexed to hexagonal HA crystals (JCPDS file No. 9-432) and no other impurity phase was detected. However, there was obvious broadening and overlap of these diffraction reflections in the XRD pattern. For example, the (211), (121), and (300) peaks of apatite were all merged into one broad peak centered at about 32°. These results implied that the as-prepared HA particles were poorly crystallized and that their grain size was on the nanometer scale.

The typical TEM image of the HA crystals is shown in Fig. 3. It was observed that the crystals had a rod-like morphology with a mean diameter and length of about 3 and 40–60 nm, respectively. Moreover, these nano-sized HA crystals exhibited good dispersity, without any severe aggregation. The phase was further identified as HA from its SAD pattern (inset of Fig. 3), wherein the polycrystalline rings for the (002) and (211) planes of HA were clearly detected. From these measurements, the precipitated particles were proven to be nano-sized HA. Also, the poor crystallization behavior and morphology of the HA crystals

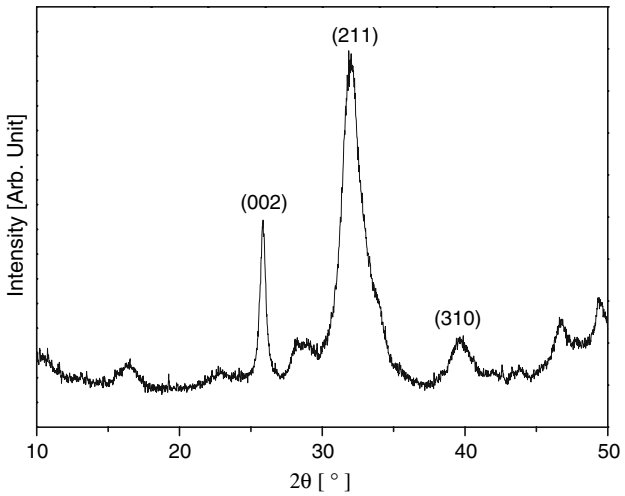

Fig. 2 X-ray diffraction pattern of the as-prepared HA precipitates

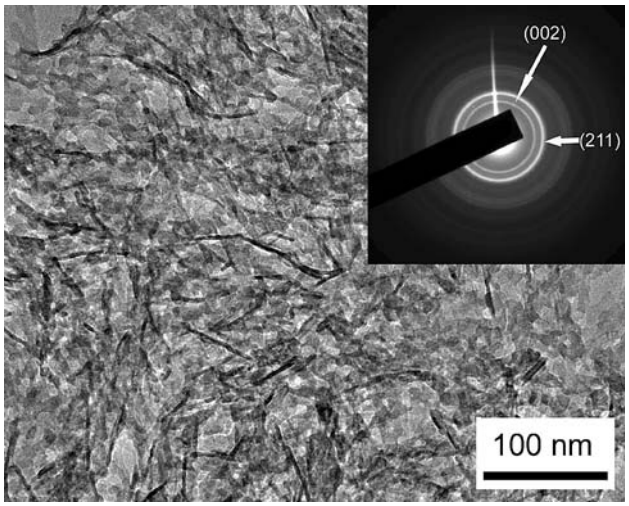

Fig. 3 TEM analysis of the precipitated HA crystals. The inset shows their selected area diffraction (SAD) pattern

prepared by the biomimetic process in this study were deemed to be very similar to those of the mineral phase in natural bone [22, 23].

3.3 Surface characterization of the coatings

The morphologies of the coatings with different compositions were observed with SEM, as presented in Fig. 4. The

surface of the pure collagen coating was quite smooth and dense (Fig. 4a), while those of the collagen/HA composite coatings became gradually rougher with increasing HA content (Fig. 4b–d). The COL-10%HA and COL-20%HA composite coatings presented good integrity and a homogeneous particle distribution, without any apparent defects. It seemed that the collagen component served, in part, as a bioadhesive that strengthened the bonding among the nano-HA particles and their adhesion to the substrates. Although the HA particles were also well dispersed in the COL-30%HA coatings, several cracks were generated on the latter, which may be due to the increase in their brittleness with increasing HA content. The typical high-magnification SEM image of the collagen/HA composite coatings in Fig. 4e clearly revealed the existence of nano-sized HA particles, which were uniformly embedded in the collagen matrix without any obvious aggregation. Fig. 4f shows the typical cross-sectional morphology of the collagen/HA composite coatings on the Ti substrates. It was observed that the coating layer was tightly bonded with the underlying Ti substrates and had a uniform thickness of approximately 7.5 μm. Moreover, there was no delamination or cracks at the interface of the coatings and the substrates, indicating the good structural integrity of the coated Ti substrates afforded by the spin coating method.

For the comparative study, a conventional composite sol containing 20 wt.% HA was prepared by mixing the collagen/HFP solution with commercial HA powder, and then spin coated on Ti substrates under the same conditions. The SEM images of the coatings that were obtained at low and high magnifications are illustrated in Fig. 5. Compared with the COL-20%HA nanocomposite coatings shown in

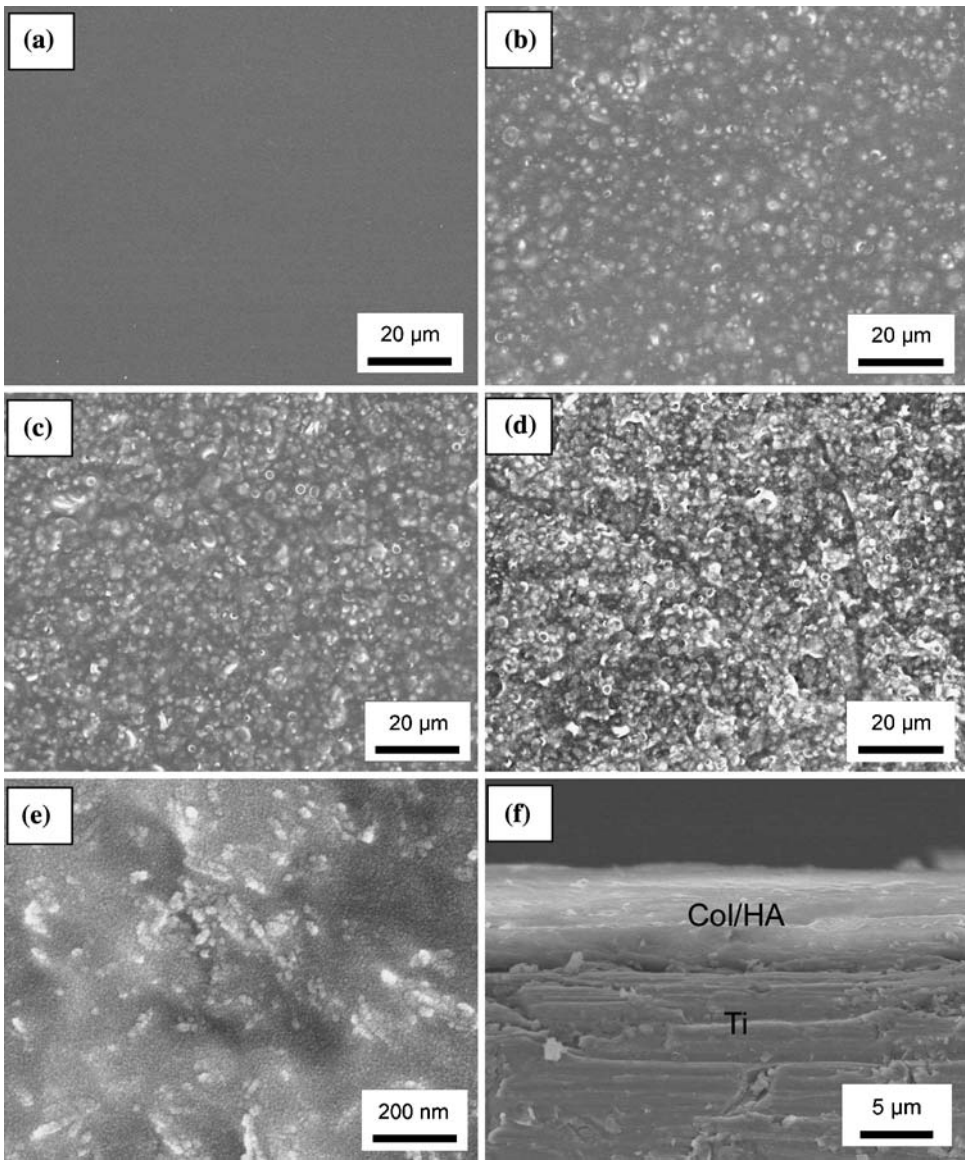

Fig. 4 SEM morphologies of the coatings with different compositions on Ti substrates: (**a**) collagen; (**b**) COL-10%HA; (**c**) COL-20%HA; (**d**) COL-30%HA; (**e**) COL-30%HA at high magnification and (**f**) its cross-section

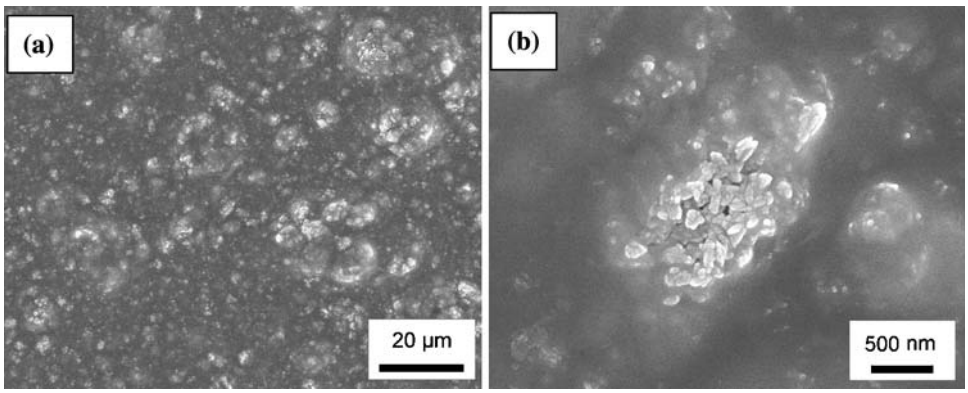

Fig. 5 The (**a**) low- and (**b**) high-magnification SEM images of the conventional composite coatings prepared by directly mixing the commercial HA powder with collagen/HFP solution

Fig. 4c, these conventional composite coatings on Ti substrates had a coarser surface, and the HA particles were severely aggregated in some regions. This result provides a good demonstration of the high efficiency of the modified mixing method in preparing uniform thin films. It is well known that nano-sized crystals are liable to agglomerate during the drying step, which no doubt significantly affects their properties and limits the use of the materials [24]. Therefore, the commercial HA powder as well as the biomimetically prepared and dried HA were not suitable to prepare a really homogeneous slurry with the collagen/HFP solution. On the other hand, the wet HA precipitates without any dry process were very easy to re-disperse and maintain in the sol state for a long period. Although a small amount of water unavoidably remained in the HA precipitates, even after high-speed centrifugation, it did not result in any obvious destruction of the sol state of the resulting composite mixture, as long as the HA content was kept at less than 50 wt.%, as mentioned above.

In the case of a biomaterial, the wettability (hydrophilicity/hydrophobicity) of its surface is considered to be one of the critical factors determining its biological performance. In this study, the wettabilities of the collagen and collagen/HA composite coatings on Ti substrates were measured based on their water contact angles. The uncoated Ti discs had a contact angle of 62.6 ± 2.1°. However, their contact angle value decreased significantly after the coating treatment. Figure 6 shows the values of the contact angles for the coatings with different compositions. Collagen itself is known to have good hydrophilic properties, showing a contact angle of 42.2°. A slight but gradual decrease in the contact angle of the composite coatings was observed with increasing HA content, viz. 41.7° for COL-10%HA and 36.5° for COL-20%HA. This suggested that the partial exposure of some of the hydrophilic groups, such as the –OH groups of HA, helps to increase the wettability of the collagen surface. However, it was reported that pure HA disks have a contact angle of about 67° [25], reflecting their more hydrophobic nature as compared to that of collagen. Therefore, when the HA content in the composite coatings was further increased up to 30 wt.%, their contact angle showed a tendency to increase again toward the value of pure HA. As shown in Fig. 6, the COL-30%HA composite coatings exhibited an average contact angle value of 40.3°, which is much higher than that of the COL-20%HA samples. It should be noted that, compared with the pure collagen coatings, the COL-30%HA composite coatings still exhibited a relatively hydrophilic surface. The variation in the contact angles of these coatings with their composition is likely to result in their having different biological performances.

3.4 In vitro evaluation of cell responses

The osteoblast responses to the collagen and collagen/HA composite coatings were assessed using MC3T3-E1 cells in terms of the cell attachment, growth and morphology. Pure Ti plates with polished surfaces were used as a control. The electron microscopy morphologies of the cells cultured on the uncoated- and coated-Ti substrates for 1 day are presented in Fig. 7. It was observed that the cells on the pure Ti substrates grew with an elongated morphology (Fig. 7a), while those cultured on the collagen coatings appeared to be more flattened (Fig. 7b) and spread in intimate contact with the underlying surface. As shown in Fig. 7c, the cells also grew favorably on the COL-30%HA composite

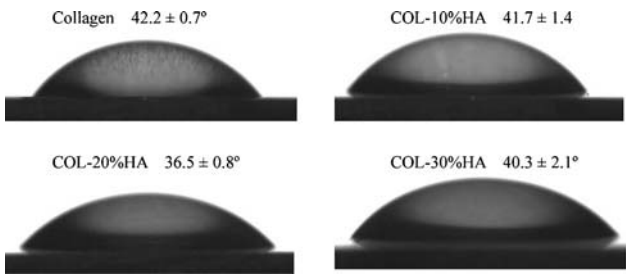

Fig. 6 Values of contact angles toward distilled water for the collagen and collagen/HA coatings on the Ti substrates. The data were represented as mean ± SD for $n = 5$

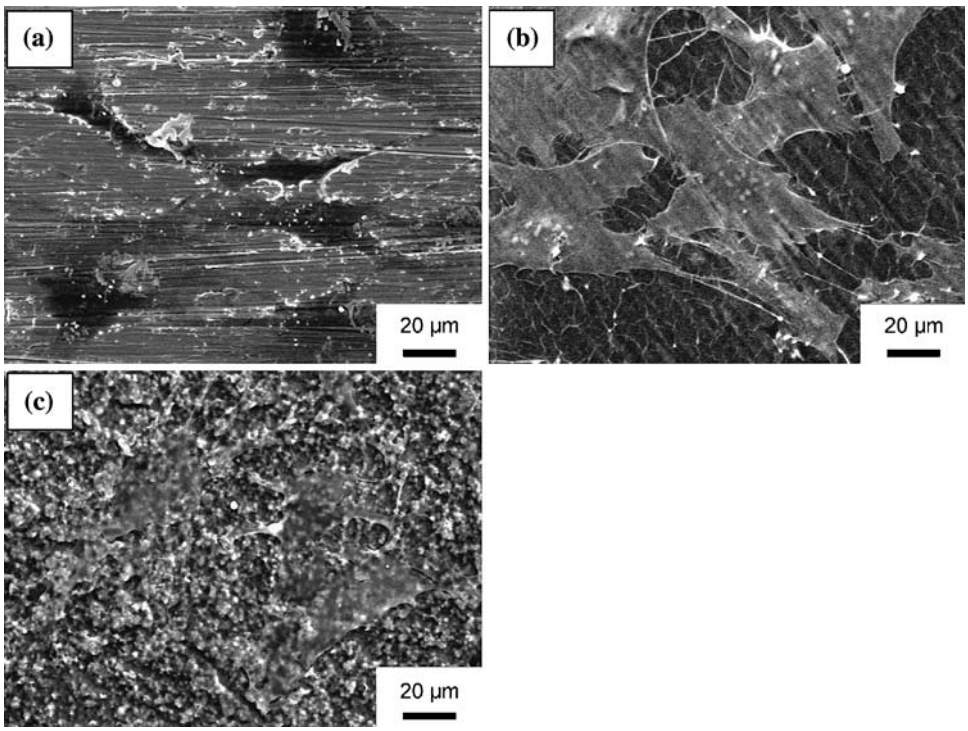

Fig. 7 The morphology of the MC3T3-E1 cells cultured on (**a**) titanium substrate, (**b**) collagen and (**c**) COL-30%HA composite coatings

coatings, achieving strong adhesion to the coarse surface by forming active cytoskeletal extensions. A similar cell growth morphology was observed on the other collagen/HA composite coatings with different compositions (figures not shown).

The cell proliferation on these coatings after 3 days of incubation is presented in Fig. 8. The MC3T3-E1 cells on the collagen-coated Ti substrates proliferated slightly better than those on the pure Ti substrates, but no significant differences existed between the two samples ($P > 0.05$). On the other hand, a significant increase in the cell proliferation level was observed in the case of the COL-10%HA and COL-20%HA composite coatings, and their significance levels with respect to the pure Ti substrates were $P < 0.05$ and $P < 0.01$, respectively. In addition, it was inferred from Fig. 8 that these two composite coatings were more favorable for cell proliferation than their pure collagen equivalents. This was probably due to improvement in the surface wettability afforded by the incorporation of HA particles, as demonstrated in Fig. 6. The enhanced osteoblastic responses of the gelatin/HA nanocomposite scaffolds as compared to those of their pure gelatin equivalents were reported by Kim et al. [26]. Accordingly, the Ti substrates coated with collagen/HA nanocomposite coatings were considered to be more promising than the collagen-coated Ti equivalents in the bone regeneration field. When comparing the nanocomposite coatings, the cells on the COL-30%HA coatings exhibited a lower proliferation level than those on the COL-20%HA coatings, and this was partially due to the relatively rougher and more hydrophobic surface of the former [27, 28].

To investigate the effect of the coating composition on the cell differentiation behavior, the ALP activity was measured by culturing MC3T3-E1 cells on both the uncoated and coated Ti substrates for up to 14 days. Even though ALP is not specific to osteoblasts, it is still widely

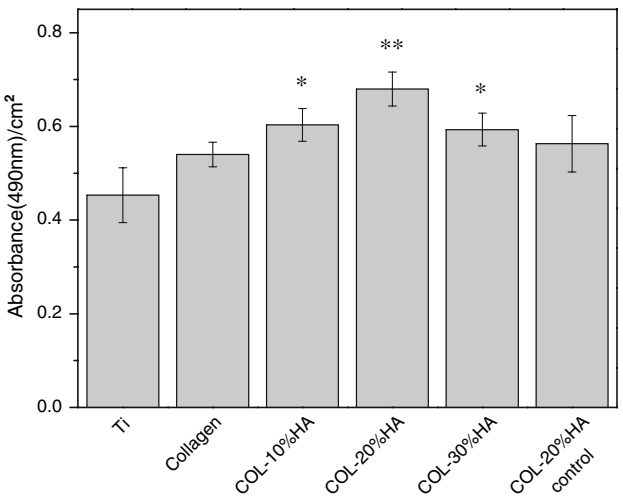

Fig. 8 Growth activity of the MC3T3-E1 cells on different coatings after 3 days. Pure Ti plates were used as a control, and differences at $*P < 0.05$ and $**P < 0.01$ with respect to pure Ti were considered statistically significant

used in vitro as a marker of the osteoblast phenotype. As could be seen from Fig. 9, both the collagen and collagen/HA composite coatings on the Ti substrates exhibited a significantly higher ALP level than the uncoated Ti plates ($P < 0.05$ for collagen and COL-10%HA; $P < 0.01$ for COL-20%HA and COL-30%HA). Compared with the collagen coatings, the COL-10%HA composite coatings showed a similar ALP activity ($P > 0.05$) while the COL-20%HA ($P < 0.05$) and COL-30%HA ($P < 0.01$) composite coatings exhibited a marked improvement in their ALP activity, illustrating the efficacy of HA in enhancing the osteoblastic phenotype expression level. The highest ALP activity was observed in the case of the COL-30%HA composite coatings, implying that these surfaces created the most favorable conditions for the expression of the osteoblast phenotype.

In addition, a comparative assessment of the cell proliferation and differentiation levels was also carried out between the COL-20%HA nanocomposite coating and the conventional composite coating with the equivalent composition. It was observed from Figs. 8 and 9 that the coatings produced by the method proposed in this study exhibited significantly higher proliferation and differentiation levels than the conventional samples. Therefore, the rough surface of the conventional composite coatings on the Ti substrates (Fig. 5), caused by the aggregation of the HA particles, was believed to retard the cell proliferation and differentiation rates, as has been reported in the case of other polymer/ceramic systems [29]. In contrast, the pure collagen and nanocomposite coatings had a relatively smooth and uniform surface, and therefore exhibited more favorable cell proliferation and differentiation behaviors.

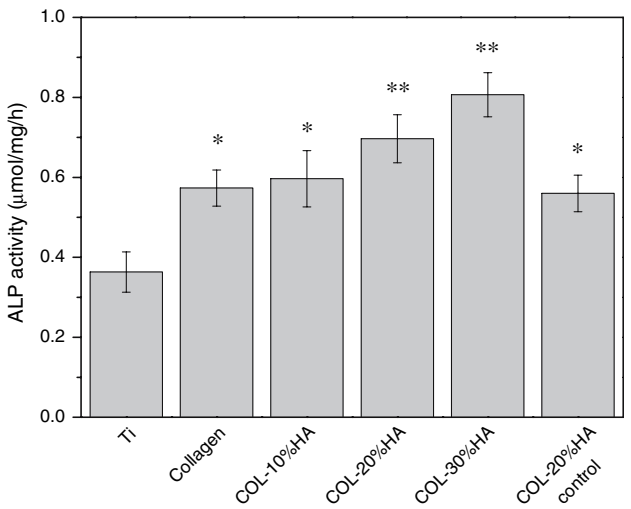

Fig. 9 Alkaline phosphatase (ALP) activity of the MC3T3-E1 cells on the uncoated and coated Ti substrates after culturing for up to 14 days (*$P < 0.05$ and **$P < 0.01$, for $n = 3$)

Based on these cellular results, we confirmed that the surface modification of Ti substrates with collagen/HA nanocomposites significantly improved the adhesion and growth of the osteoblastic MC3T3-E1 cells and induced them to differentiate at an enhanced level. Moreover, the collagen/HA nanocomposite coatings were observed to exhibit much better cell proliferation behaviors and osteogenic potentials than the conventional composite coatings with equivalent compositions, suggesting that the collagen/HA nanocomposite coatings obtained biomimetically on the Ti substrates have greater potential for use as an implant material for hard tissue engineering. Further evaluations on the biodegradability of these coatings and in vivo tissue responses are currently underway.

4 Conclusions

In this study, a homogeneous collagen/HA nanocomposite sol was obtained by the biomimetic preparation and centrifugation of nano-sized HA precipitates followed by mixing with collagen without any dry treatment. Subsequently, collagen/HA composite thin coatings with an approximate thickness of 7.5 μm were uniformly formed on Ti substrates through a spin coating process. The SEM results showed that the nano-sized HA particles were well distributed within the collagen matrix, without any obvious aggregation. When the content of HA reached 20 wt.%, the nanocomposite coatings exhibited the lowest contact angle of 36.5° and the highest proliferation rate of the osteoblast-like MC3T3-E1 cells. As compared to both the bare Ti substrates and the conventional composite coatings with equivalent compositions, the collagen/HA nanocomposite coatings on the Ti substrates obtained with the present method showed much better cell proliferation behaviors and osteogenic potentials, thus confirming the improved activity of the cell functions that they afford. In conclusion, collagen/HA nanocomposites have great potential for use as a coating material for future medical applications in hard and soft tissue replacements.

Acknowledgements This work was supported by a grant from the Korea Health 21 R&D Project, the ministry of Health & Welfare, Republic of Korea (02-PJ3-PG6EV11-0002).

References

1. H. Tschernitschek, L. Borchers, W. Geurtsen, Quintessence Int. **36**, 523 (2005)
2. A.M. Al-Mayouf, A.A. Al-Swayih, N.A. Al-Mobarak, A.S. Al-Jabab, Mater. Chem. Phys. **86**, 320 (2004)
3. E.J. Mcpherson, L.D. Dorr, T.A. Gruen, M.T. Saberi, Clin. Orthop. **315**, 223 (1995)

4. I.S. Lee, D.H. Kim, H.E. Kim, Y.C. Jung, C.H. Han, Biomaterials **23**, 609 (2002)
5. S. Overgaard, M. Lind, H. Glerup, S. Grundvig, C. Bunger, K. Soballe, Clin. Orthop. Relat. Res. **336**, 286 (1997)
6. J.G.C. Wolke, J.P.C.M. Van Der Waerden, H.G. Schaeken, J.A. Jansen, Biomaterials **24**, 2623 (2003)
7. J.L. Ong, M. Appleford, S. Oh, Y. Yang, W.H. Chen, J.D. Bumgardner, W.O. Haggard, JOM **58**, 67 (2006)
8. X.B. Zheng, C.X. Ding, J. Therm. Spray Technnol. **9**, 520 (2007)
9. T. Furuzono, A. Kishida, J. Tanaka, J. Mater. Sci.: Mater. Med. **15**, 19 (2004)
10. A. Afshar, M. Yousefpour, X. Yang, X. Li, B. Yang, Y. Wu, J. Chen, X. Zhang, Mater. Sci. Eng. B **128**, 243 (2006)
11. H. W. Kim, L.H. Li, E.J. Lee, S.H. Lee, H.E. Kim, J. Biomed. Mater. Res. **75A**, 629 (2005)
12. S. Rammelt, T. Illert, S. Bierbaum, D. Scharnweber, H. Zwipp, W. Schneiders, Biomaterials **27**, 5561 (2006)
13. S. Rammelt, E. Schulze, R. Bernhardt, U. Hanisch, D. Scharnweber, H. Worch, H. Zwipp, A. Biewener, J. Orthop. Res. **22**, 1025 (2004)
14. M. Morra, C. Cassinelli, L. Meda, M. Fini, G. Giavaresi, R. Giardino, Int. J. Oral Maxillofac. Implants **20**, 23 (2005)
15. S. Li, Z. Zheng, W. Liu, J.R. De Wijin, K. De Groot, J. Biomed. Mater. Res. **40**, 520 (1998)
16. Y. Cai, C. Liang, S. Zhu, Z. Cui, X. Yang, Scripta Mater. **54**, 89 (2006)
17. K. Hu, X.J. Yang, Y.L. Cai, Z.D. Cui, Q. Wei, Surf. Coat Technol. **201**, 1902 (2006)
18. Y. Chen, A.F.T. Mak, M. Wang, J. Li, J. Biomed. Mater. Res. **77B**, 315 (2006)
19. Y. Chen, A.F.T. Mak, M. Wang, J. Li, M.S. Wong, Surf. Coat Technol. **201**, 575 (2006)
20. S. Yokota, T. Kitaoka, H. Wariishi, Appl. Surf. Sci. **253**, 4208 (2007)
21. K.H. Wu, S.Y. Lu, H.L. Chen, Langmuir **22**, 8029 (2006)
22. X. Lin, X. Li, H. Fan, X. Wen, J. Lu, X. Zhang, Mater. Lett. **58**, 3569 (2004)
23. D.D. Lee, M.J. Glimcher, J. Mol. Biol. **217**, 487 (1991)
24. A. Tampeeri, G. Celotti, F. Szontagh, E. Landi, J. Mater. Sci.: Mater. Med. **8**, 29 (1997)
25. A. Monkawa, T. Ikoma, S. Yunoki, K. Ohta, J. Tanaka, J. Nanosci. Nanotechnol. **7**, 833 (2007)
26. H.W. Kim, H.E. Kim, V. Salih, Biomaterials **26**, 5221 (2005)
27. P. Linez-Bataillon, F. Monchau, M. Bigerelle, H.F. Hildebrand, Biomol. Eng. **19**, 133 (2002)
28. J.H. Lee, G. Khang, J.W. Lee, H.B. Lee, J. Colloid Interf. Sci. **205**, 323 (1998)
29. H.W. Kim, J.C. Knowles, H.E. Kim, J. Biomed. Mater. Res. **72A**, 136 (2004)