

Growth of nano-scale hydroxyapatite using chemically treated titanium oxide nanotubes

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Abstract

A vertically aligned nanotube array of titanium oxide was fabricated on the surface of titanium substrate by anodization. The nanotubes were then treated with NaOH solution to make them bioactive, and to induce growth of hydroxyapatite (bone-like calcium phosphate) in a simulated body fluid. It is shown that the presence of TiO₂ nanotubes induces the growth of a “nano-inspired nanostructure”, i.e., extremely fine-scale (~8 nm feature) nanofibers of bioactive sodium titanate structure on the top edge of the ~15 nm thick nanotube wall. During the subsequent in-vitro immersion in a simulated body fluid, the nano-scale sodium titanate, in turn, induced the nucleation and growth nano-dimensioned hydroxyapatite (HAp) phase. The kinetics of HAp formation is significantly accelerated by the presence of the nanostructures. Such TiO₂ nanotube arrays and associated nanostructures can be useful as a well-adhered bioactive surface layer on Ti implant metals for orthopaedic and dental implants, as well as for photocatalysts and other sensor applications.

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1. Introduction

Ti and Ti alloys are corrosion resistant, light, yet sufficiently strong for use as load-bearing and machinable orthopaedic implant materials. They are one of the few biocompatible metals which osseointegrate (direct chemical or physical bonding with adjacent bone surface without forming a fibrous tissue interface layer). For these reasons, they have been used successfully as orthopaedic and dental implants [1,2]. To impart bioactivity to Ti and enhance bone growth, surface treatments such as surface roughening by sand blasting, formation of anatase phase TiO₂ [3], hydroxyapatite (HAp) coating, or chemical treatment [4–8] have been utilized. Kim et al. [8] reported that HAp formation

from Ti metal surface can be accelerated by the introduction of sodium titanate layer when titanium surface is subjected to NaOH treatment followed by heat treatment. Webster et al. [9,10] reported that a creation of nanostructure on a ceramic material such as aluminum oxide with grain/particle size of less than ~100 nm regime significantly improved bioactivity of implant and enhanced osteoblast adhesion.

Adhesion of cells such as osteoblasts is an important prerequisite to subsequent cell functions.

TiO₂ nanostructures have received considerable attention in recent years as photo-excited TiO₂ also exhibits strong photocatalytic properties and offers some potentially important technical applications. Titanium oxide nanotubes can be prepared by various techniques such as sol–gel method [11], electrophoretic deposition [12] and anodization [13]. For bio-implant applications, the adhesion and mechanical integrity of the TiO₂ layer are important. Anodization is preferred to the sol–gel and

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electrophoretic deposition as it provides strongly adherent TiO₂ layer that the other two approaches generally do not produce. While the fabrication of vertically aligned TiO₂ nanotubes on Ti substrate was demonstrated by anodization process [13], an investigation of such nanotubes for bone growth studies has not been reported. Such an aligned TiO₂ nanotube structure would be useful especially since it can be made as a tightly adherent surface layer on Ti metal surface. The purpose of this research work is to study the microstructural features and the effect of vertically aligned TiO₂ nanotubes and associated nanostructures on *in vitro* HAp formation kinetics and morphology.

2. Materials and methods

Titanium oxide nanotubes were fabricated using the anodization technique. The anodization process involved is as follows; a titanium sheet (0.25 mm thick, 99.5% purity), was procured from Alfa AESAR, Ward Hill, MA, cut into small pieces (2.54 cm × 1.27 cm), chemically cleaned/etched for 5 min in 5.5 M of HNO₃ with a few drops of HF (ACS grade, Fisher Scientific, Pittsburg, PA), rinsed by distilled water and dried at 40 °C. The electrolyte solution for anodization process consisted of 0.5% by weight of HF in water. A platinum electrode (thickness: 0.1 mm, purity: 99.99%, Alfa Aesar, Ward Hill, MA) was used as the cathode. The TiO₂ nanotubes were prepared by using a 20 V anodization voltage and for up to 30 min of anodization time at room temperature. The titanium oxide nanotubes so produced were then chemically treated with NaOH solution to explore the possibility of enhancing the bioactivity of the titanium oxide nanotubes as observed in the case of metallic Ti exposed to NaOH [8,14–16]. For chemical treatment, the nanotube samples were immersed in a 5 mol NaOH solution at ~60 °C for up to 60 min. The nanotube samples were then heat treated at 500 °C/2 h to crystallize the amorphous TiO₂ nanotubes into the anatase structure.

For evaluation of bone growth on bioactive surface in terms of HAp formation, the TiO₂ nanotube specimens were soaked for 1, 2, 3 and 5 days, in 20 mL of a simulated body fluid (SBF) solution at 36.5 °C, which contained ion concentrations nearly equal to those of human blood plasma [17] with respect to Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, HCO₃⁻, HPO₄²⁻, and SO₄²⁻ concentrations. After a predetermined soaking time of 1, 2, 3 and 5 days, the specimens were removed from the SBF solution, gently rinsed with distilled water, and then dried at 60 °C for 24 h. Microstructural/morphological changes of the dried specimen surfaces were examined by scanning electron microscopy (SEM: Quanta 600, FEI company), energy dispersive X-ray analysis (EDXA) in SEM, transmission electron microscopy

(JEOL 200CX operated at 200 kV), and thin film X-ray diffractometer (DMAX 2000, Rigaku Corp.).

3. Results and discussion

The SEM micrographs showing the evolution of microstructure during anodization of the Ti sheet substrate are shown in Fig. 1. It is interesting to see that various types of substantially different nanostructures of TiO₂ can be synthesized. For a very short anodizing time of just ~1 min, TiO₂ nanodots are formed, Fig. 1(a). After 15 min of anodizing time, TiO₂ nanowires are produced, Fig. 1(b). On a longer anodizing time of 30 min, the nanowires are transformed into another geometry of TiO₂ nanotubes. The nanotubes are grown in a vertically aligned, parallel configuration as shown in Fig. 1(c). A higher magnification SEM (Fig. 2) and a TEM micrograph, Fig. 3, indicate that the typical dimensions of the hollow nanotubes grown in this study is ~100 nm outer diameter, ~70 nm inner diameter, ~15 nm wall thickness, and ~250 nm in height. The nanotubes as fabricated by anodization was found to be amorphous as evidenced by the presence of a very diffuse diffraction pattern in thin film X-ray diffraction analysis carried out at a glancing angle of 1°. Subsequent heat treatment at 500 °C crystallizes the amorphous nanotubes into the anatase structure as discussed earlier. The formation mechanism of TiO₂ nanotubes was discussed by Mor et al. [18].

It is well established that the anatase phase TiO₂ is much more efficient in nucleation and growth of HAp (bone growth) than the rutile phase TiO₂ presumably because of the better lattice match with HAp phase [3]. Annealing heat treatment of the amorphous TiO₂ material by continuous heating to ~500 °C produces an anatase crystal structure of TiO₂, shown in Fig. 2(b), which is not much different from the as-anodized structure of Fig. 2(a). Heating to a higher temperature in excess of 600 °C generally results in the undesirable rutile structure and some collapse of the TiO₂ nanotubes structure [19]. A top-view TEM micrograph of the annealed and anatase-structured TiO₂ nanotubes is given in Fig. 3. The nanotubes, less than ~100 nm in average diameter, have a hollow core and are slightly separated from each other. Electron diffraction pattern in the inset shows somewhat regular pattern-like feature which implies that some crystallographic orientation relationship exists among the various parallel TiO₂ nanotubes.

On exposure of the TiO₂ nanotubes to the NaOH solution to improve bioactivity, it has been found that an additional, extremely fine, and predominantly nanofiber-like structure (or in some cases a nanoribbon-like structure) is introduced on the very top of the

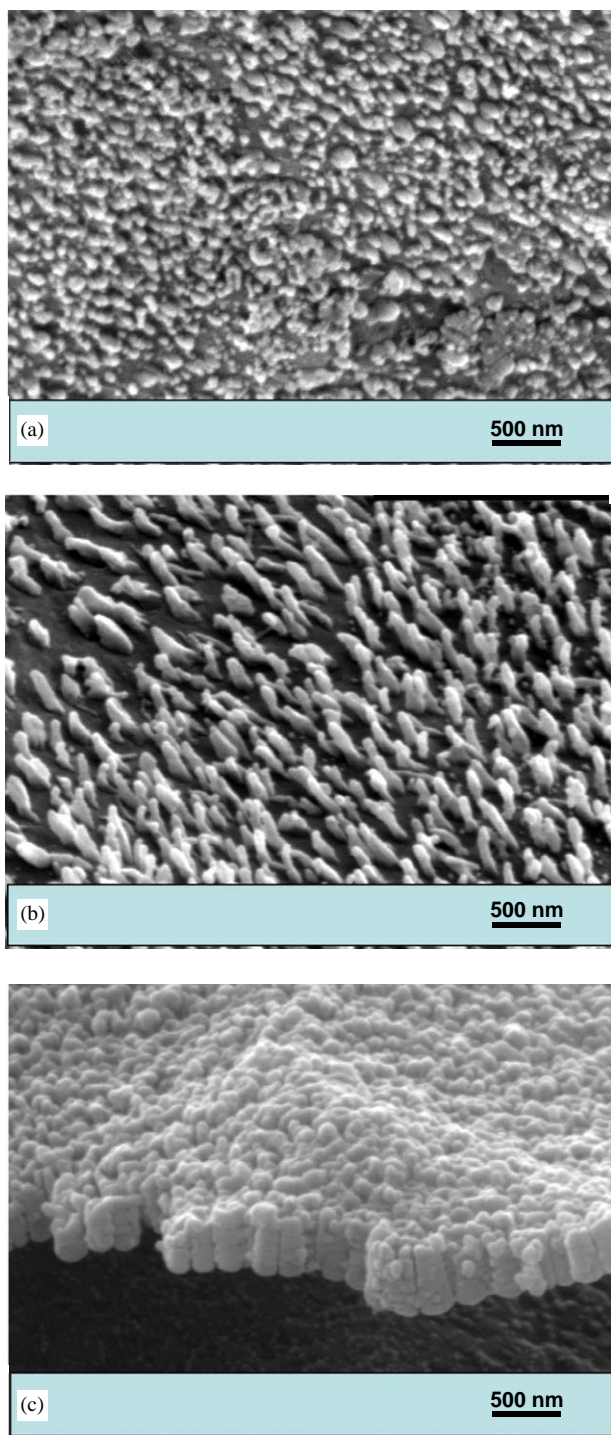


Fig. 1. SEM micrographs showing the microstructure during anodization of Ti substrate after (a) 1 min, (b) 15 min, (c) 30 min.

TiO₂ nanotubes as shown in Fig. 4(a). These nanofibers are most likely to be the sodium titanate compound similar to that reported in the case of the reaction product on exposure of metallic Ti to NaOH. The preferential occurrence of nanofibers at the top of nanotubes is presumably because of the nanotube

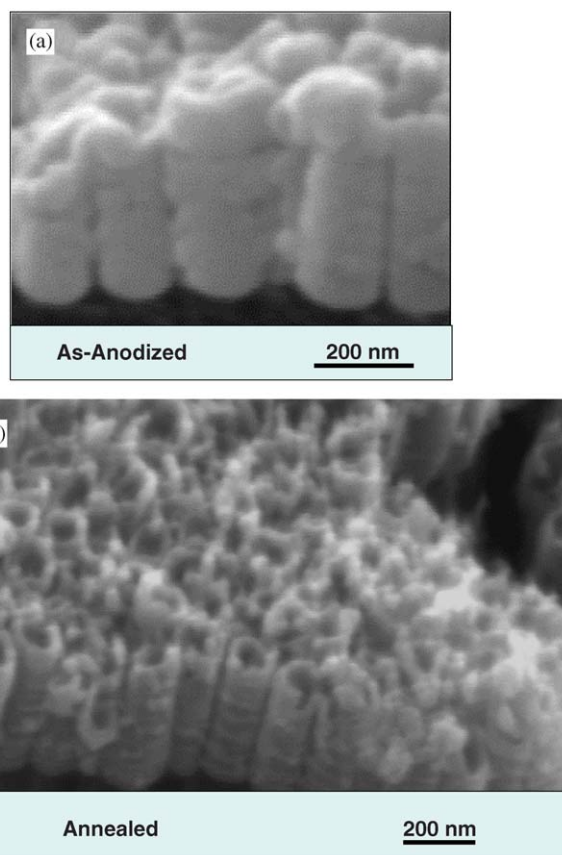


Fig. 2. SEM microstructure of TiO₂ nanotubes; (a) as-anodized (b) after annealing.

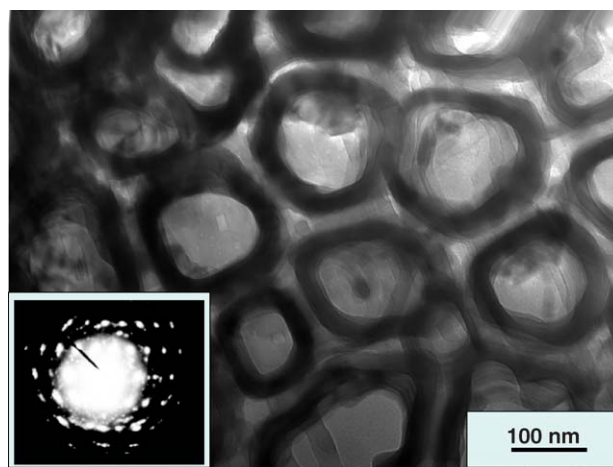


Fig. 3. Top-view TEM micrograph of the annealed TiO₂ nanotubes.

contact with NaOH solution above and also possibly due to the surface-tension-related difficulty of NaOH solution getting into nanopores within and in-between TiO₂ nanotubes. Compositional analysis by EDXA in SEM, Fig. 5, indicates the presence of Na, Ti and O after the exposure of TiO₂ nanotubes to NaOH, thus

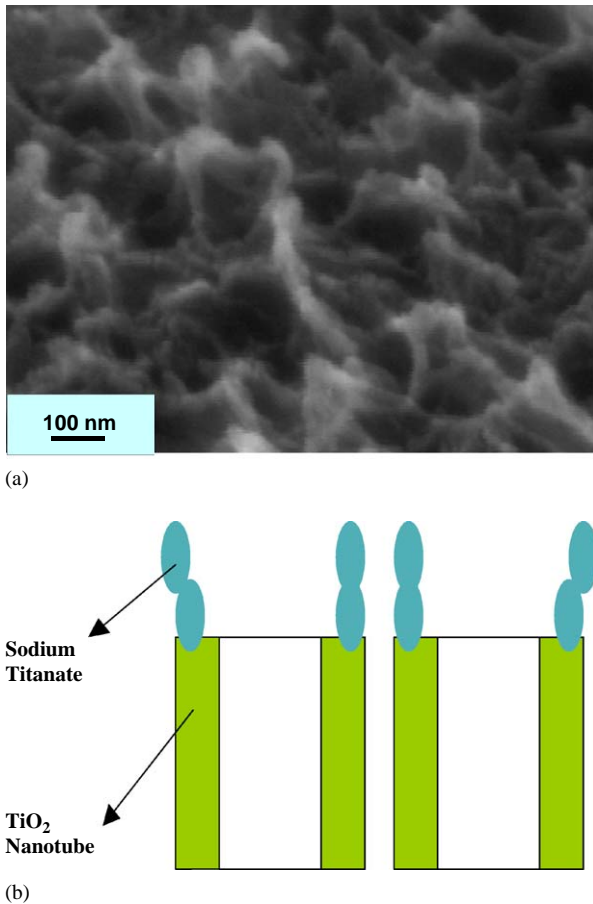


Fig. 4. Nanostructure of TiO₂ nanotubes; (a) SEM micrograph showing the sodium titanate nanofiber structure on nanotubes, (b) a schematic illustration of the formation of nano-inspired nanostructure.

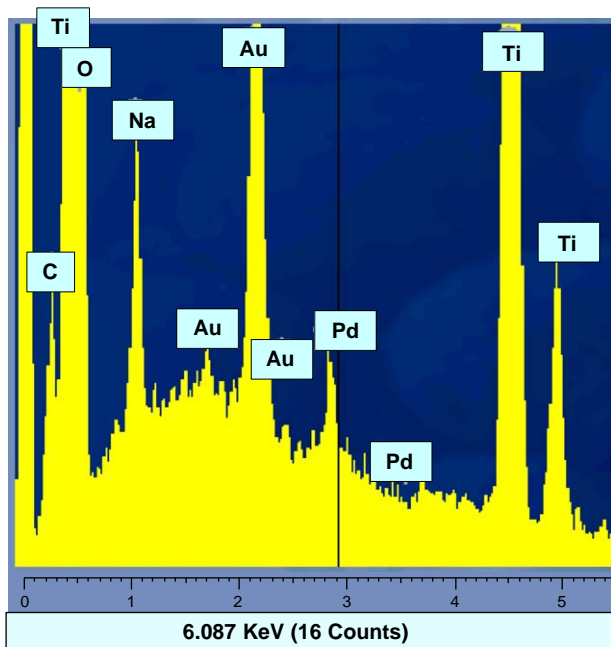


Fig. 5. EDXA analysis data showing the presence of Na, Ti and O in the sodium titanate nanofiber structure.

supporting the formation of sodium titanate. The presence of Au and Pd peaks is due to the sample metallization for SEM analysis via sputtering deposition from an Au–Pd alloy target. The sodium titanate (which is likely to have a composition of Na₂Ti₅O₁₁ or Na₂Ti₆O₁₃ [14]) so introduced exhibits an extremely fine-scale nanofiber configuration with a dimension of ~8 nm in average diameter and ~50–100 nm long. What is noteworthy is that the nanofibers appear to form on the upper edge of the nanotube wall, as is schematically illustrated in Fig. 4(b). This is experimentally confirmed by SEM of Fig. 4(a) and TEM of Fig. 6.

The growth of even finer-scale structure from a given nanostructure as demonstrated in this work can be of significant interest for basic materials development for nanotechnology, since such a concept can be utilized as one of the novel and efficient ways of creating extremely fine nanostructures in many different materials. It is believed that the nanofiber-shaped sodium titanate phase is formed in such a fine scale because of the physically confined geometry of the host structure, TiO₂ nanotubes. Since the nucleation and growth of the sodium titanate phase occurs on TiO₂ which has the ring-shaped end material facing outward with the tube wall thickness of only ~15 nm, the sodium titanate phase growing from the host surface is likely to be in the order of or less than this dimension, as is actually observed. We tentatively call such a mechanism as “Nano-inspired Nanostructure”. The process of forming nano-inspired nanostructure can also be viewed as a hierarchical construction of nanostructure, which can be important for nanostructural engineering, for example, for creation of catalyst structures with ultra-large surface area.

The formation of bone-growth-related material such as the calcium phosphate mineral, HAp (Ca₁₀(PO₄)₆(OH)₂), is an important issue for orthopaedic and dental implants. Bone is a calcium phosphate-based mineral which contains ~70% HAp-like material with

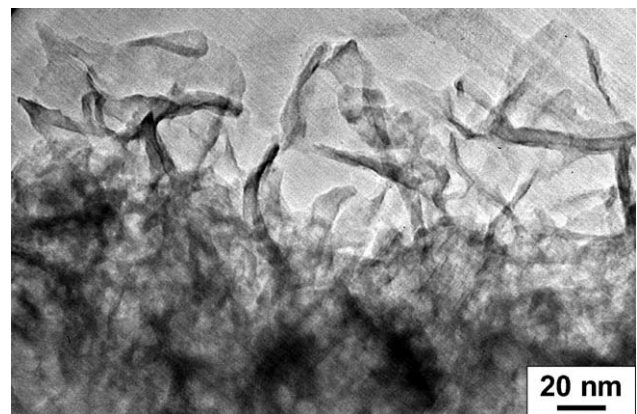


Fig. 6. TEM micrograph showing the sodium titanate nanofiber structure.

the remainder consisting mostly of collagen. In order to enhance the formation of such calcium phosphate minerals, the effects of nanostructure formation are investigated in this work by exposing the nanostructure of Figs. 4 and 6 to a simulated body solution, and the morphology and kinetics of HAp growth have been studied.

Sodium titanate which is amorphous in the NaOH treated state is well known for accelerating HAp formation in SBF solution as well as in animal body [20–22]. In this work, the TiO₂ nanotube surface covered with “Nano-inspired Nanostructure” of sodium titanate was subjected to the SBF solution. The SEM microstructure of the sample after the SBF soaking for 1 day (SBF soaking for longer periods of 2–5 days results in a complete coverage of the sample surface) is shown in Fig. 7. It is seen that the sample surface is basically covered with HAp-like structure, with the EDXA spectrum in Fig. 8 clearly showing the presence of Ca, P, and O, the essential ingredients of HAp. X-ray diffraction analysis also gave similar results. The formation of HAp in the TiO₂ nanotube surface containing sodium titanate nanofibers is significantly accelerated as compared with the same TiO₂ nanotube surface but without the sodium titanate nanofibers. In the latter case, it took ~7 days for formation of detectable amount of HAp, as compared with just 1 day for the sample covered with sodium titanate nanofibers. As is evident from Fig. 7, the HAp formed is by itself nanostructured with a nanofiber morphology resembling that of the sodium titanate. The nanofiber feature size of the HAp phase formed is ~25 nm average diameter. It appears that the nanofiber HAp (Fig. 7) nucleated and grew from the nanofiber sodium titanate precursor (Fig. 4). The ~25 nm average diameter of the nanofiber HAp is somewhat coarser than its precursor sodium titanate (~8 nm) as might be anticipated for the extended (1 day) exposure to SBF. The nanofiber HAp

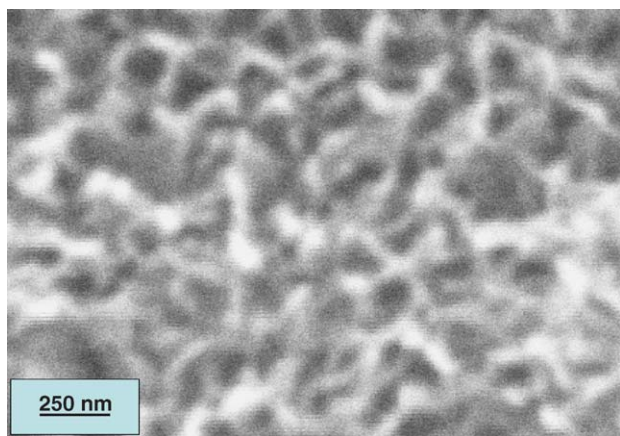


Fig. 7. SEM microstructure of the nanoscale HAp phase formed on chemically treated TiO₂ nanotubes.

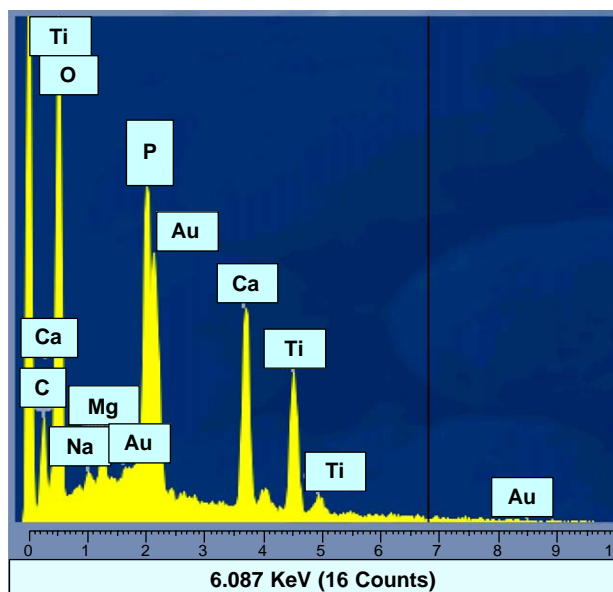


Fig. 8. EDXA spectrum showing the presence of Ca and P in the HAp phase.

obtained in this work is, to the best of our knowledge, the smallest feature HAp reported so far.

HAp can be formed easily in the presence of sodium titanate by the ion exchange between Na⁺ on the host structure and Ca²⁺ in the SBF solution [23]. It is likely that several factors play a critical role in HAp formation on the surface of implant materials in SBF solution, such as the surface area and roughness [24], electrical charge [23] of the host substrate as well as the concentration [25] and pH [26] of the SBF solution. Further research is needed to obtain more detailed understanding of the effect of nanostructured host material on kinetics, morphology, and other specific details of HAp formation.

According to Webster et al. [9,10], an introduction of nanostructure significantly improves osteoblast adhesion. Adhesion of cells such as osteoblasts is a crucial prerequisite to subsequent cell functions such as synthesis of extracellular matrix proteins, and formation of mineral deposits. Our experimental observation of enhanced HAp formation on nanostructured TiO₂ is therefore basically in agreement with the trend that Webster et al.'s results indicated from the view point of overall enhanced bone formation, although the exact relationship between the enhanced osteoblast adhesion and the enhanced HAp formation is yet to be fully understood. The base nanostructure in our work is about one order of magnitude smaller in feature size than in Webster et al.'s work. It would be interesting how such an extremely fine nanostructure influences the cell behavior. The effect of such a structure on the behavior of osteoblast cells in our samples is currently being investigated. The preliminary results seem to

indicate that the enhanced osteoblast adhesion and the enhanced HAp formation occur simultaneously. The detailed results will be reported in future publications.

4. Conclusions

A vertically aligned nanotube array of titanium oxide on the surface of titanium substrate has been prepared by anodization, which was then treated with NaOH solution to make them bioactive. It is shown that the presence of TiO₂ nanotubes induces the growth of a “nano-inspired nanostructure”, i.e., extremely fine-scale nanofibers of bioactive sodium titanate structure on the top edge of the ~ 15 nm thick nanotube wall. In-vitro immersion of the nanotube structure in a simulated body fluid induced the nucleation and growth of nano-dimensioned hydroxyapatite (HAp) phase. The kinetics of HAp formation is significantly accelerated by the presence of the nanostructure. Such TiO₂ nanotube arrays and associated nanostructures can be useful as well-adhered bioactive surface layers on Ti implant metals and alloys for orthopaedic and dental applications.

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References

- [1] Black J, Hasting G, editors. Handbook of biomaterial properties. London: Chapman & Hall; 1998 p. 179–200.
- [2] Ratner BD, Hoffman AS, Schoen FJ, Lemons JE. Biomaterials science. San Diego, CA: Academic Press; 1996.
- [3] Uchida M, Kim HM, Kokubo T, Fujibayashi S, Nakamura T. Structural dependence of apatite formation on titania gels in a simulated body fluid. *J Biomed Mater Res* 2003;64:164–70.
- [4] Lugscheider E, Weber T, Knepper M, Vizethum F. Production of biocompatible coatings by atmospheric plasma spraying. *Mater Sci Eng A: Struct Mater: Properties Microstruct Proc* 1991;A139:45–8.
- [5] Ducheyne P, Van Raemdonck W, Heughebaert JC, Heughebaert M. Structural analysis of hydroxyapatite coatings on titanium. *Biomaterials* 1986;7(2):97–103.
- [6] Cooley DR, Van Dellen AF, Burgess JO, Windeler AS. The advantages of coated titanium implants prepared by radio-frequency sputtering from hydroxyapatite. *J Prosthet Dent* 1992;67(1):93–100.
- [7] De Andrade MC, Sader MS, Filgueiras MRT, Ogasawara T. Microstructure of ceramic coating on titanium surface as a result of hydrothermal treatment. *J Mater Sci: Mater Med* 2000;11(11):751–5.
- [8] Kim HM, Miyaji F, Kokubo T, Nakamura T. Preparation of bioactive Ti and its alloys via simple chemical surface treatment. *J Biomed Mater Res* 1996;32(3):409–17.
- [9] Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Enhanced functions of osteoblast on nanophase ceramics. *Biomaterials* 2000;21:1803–10.
- [10] Webster TJ, Schandler LS, Siegel RW, Bizios R. Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin. *Tissue Eng* 2001;7:291–301.
- [11] Lakshmi BB, Patrissi CJ, Martin CR. Sol–gel template synthesis of semiconductor oxide micro- and nanostructures. *Chem Mater* 1997;9:2544–50.
- [12] Miao Z, Xu D, Ouyang J, Guo G, Zhao X, Tang Y. Electrochemically induced sol–gel preparation of single-crystalline TiO₂ nanowires. *Nano Lett* 2002;2(7):717–20.
- [13] Gong D, Grimes CA, Varghese OK, Hu W, Singh RS, Chen Z, Dickey EC. Titanium oxide nanotube arrays prepared by anodic oxidation. *J Mater Res* 2001;16(12):3331–4.
- [14] Lee BH, Kim YD, Shin JH, Lee KH. Surface modification by alkali and heat treatments in titanium alloys. *J Biomed Mater Res* 2002;61(3):466–73.
- [15] Kokubo T, Kim HM, Kawashita M. Novel bioactive materials with different mechanical properties. *Biomaterials* 2003;24(13):2161–75.
- [16] Takadama H, Kim HM, Kokubo T, Nakamura T. TEM-EDX study of mechanism of bonelike apatite formation on bioactive titanium metal in simulated body fluid. *J Biomed Mater Res* 2001;57(3):441–8.
- [17] Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T. Solution able to reproduce in-vivo surface-structure changes in bioactive glass-ceramic. *J Biomed Mater Res* 1990;24:721–34.
- [18] Mor GK, Varghese O. Fabrication of tapered, conical-shaped titania nanotubes. *J Mater Res* 2003;18:2588–93.
- [19] Varghese O, Gong D, Paulose M, Grimes C, Dickey E. Crystallization and high-temperature structural stability of titanium oxide nanotube arrays. *J Mater Res* 2003;18:156–65.
- [20] Chen M, Yang X, He F, Liu Y, Zhu S, Cui Z. Effect of NaOH concentration on formation of bone-like apatite layer on NiTi shape memory alloy. *Jinshu Xuebao* 2003;39(8):859–64.
- [21] Wang XX, Hayakawa S, Tsuru K, Osaka A. A comparative study of in vitro apatite deposition on heat-, H₂O₂-, and NaOH-treated titanium surfaces. *J Biomed Mater Res* 2001;54(2):172–8.
- [22] Uchida M, Kim HM, Kokubo T, Fujibayashi S, Nakamura T. Effect of water treatment on the apatite-forming ability of NaOH-treated titanium metal. *J Biomed Mater Res* 2002;63(5):522–30.
- [23] Kim HM, Himeno T, Kawashita M, Lee JH, Kokubo T, Nakamura T. Surface potential change in bioactive titanium metal during the process of apatite formation in simulated body fluid. *J Biomed Mater Res* 2003;67A(4):1305–9.
- [24] Ding S, Ju C, Lin JC. Immersion behavior of RF magnetron-assisted sputtered hydroxyapatite/titanium coatings in simulated body fluid. *J Biomed Mater Res* 1999;47(4):551–63.
- [25] Barrere F, van Blitterswijk CA, de Groot K, Layrolle P. Nucleation of biomimetic Ca-P coatings on Ti₆Al₄V from a SBF solution: influence of magnesium. *Biomaterials* 2002;23(10):2211–20.
- [26] Chou YF, Chiou WA, Xu Y, Dunn JCY, Wu BM. The effect of pH on the structural evolution of accelerated biomimetic apatite. *Biomaterials* 2004;25(22):5323–31.