In situ synthesis of hydroxyapatite-grafted titanium nanotube composite

Mostafa Rezazadeh Shirdara, Mohammad Mahdi Taheria,b, Izman Sudina, Arezou Shafaghatc, Ali Keyvanfar c and Muhd Zaimi Abd. Majidc

aDepartment of Materials, Manufacturing and Industrial Engineering, Faculty of Mechanical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia; bMedical Devices & Technology Group, Faculty of Bioscience & Medical Engineering, Universiti Teknologi Malaysia, Skudai, Malaysia; cUTM Construction Research Centre, Institute for Smart Infrastructure and Innovative Construction, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia

ABSTRACT
The present study is an investigation to demonstrate the effectiveness of in situ approach in the synthesis of hydroxyapatite-grafted titanium nanotube composite (HA-TNT). This method involves combining the process of HA sol—gel and rapid breakdown anodisation of titanium in a novel solution consisting of NaCl and N3PO4. This new synthesis approach produced a uniform dispersion of Anatase and Rutile phases of TiO2 nanotubes with minimal agglomeration in the matrix of crystalline HA. The characterisation of homogenised HA-TNT composite was investigated via field emission scanning electron microscopy (FESEM), energy dispersive spectroscopy (EDS), transmission electron microscope (TEM) and X-ray diffraction (XRD). FESEM and TEM images indicated the nanostructure of composite with TiO2 nanotube diameter of approximately 10 nm. XRD and EDS analyses confirmed the formation of HA crystalline with the Ca/P ratio of 1.58 and formation of Anatase and Rutile phase of TiO2 nanotubes.

1. Introduction
Nowadays, considerable attentions have been drawn to the application of hydroxyapatite (HA: Ca10 (PO4)6(OH) 2) in biomedical fields. This is due to the similarity of natural bone to HA in terms of structural and chemical compositions. [1—3] Due to its bioactivity and biocompatibility, HA has been widely used as an alternative material for the damaged teeth or bone over the past three decades. [4—7] HA can be synthesised through various techniques such as hydrothermal, sol—gel, precipitation, electrodeposition and biometric deposition. [8—10] Among these techniques, sol—gel is highly preferred due to its homogeneity, low synthesis temperature and possibility of forming nanosized particles. [11] As it has been recorded, the mixtures of nano or submicron particles are efficient in improving osseointegration in vitro and vivo environments. Usually, sol—gel technique is
utilised to synthesise the nano-to-micro particles of HA in crystal structures. [12,13] However, HA ceramic suffers relatively poor mechanical properties which impede its usage in long-term load-carrying applications. [14,15] Some attempts have been made to enhance the mechanical properties of HA by implementing fillers such as bio-inert ceramics like alumina, [16] zirconia [17] and titania [18] as well as other metals, polymer fibres and bioglass [19] in the HA matrix.

Among the diverse HA-based composites, HA/TiO2 composites have drawn impres-sive consideration recently. This is due to the assumption that titania has a great capability to improve osteoblast bond, and it can incite cell development. [20] It has been also demonstrat-ed that cohesive and adhesive strength of HA implant significantly increased by adding titania as the reinforcement. [21] In addition, Xiao et al. have reported that the incorporation of TiO2 in the matrix of HA improved the corrosion resistance of pure HA. [22] Moreover, several studies have demonstrated that Titanium nanotube is bio-inert and biocompatible in nanoscale; therefore, it could be useful for bioapplications. [23] Furthermore, the presence of the nanotube structure induced a significant acceleration in the growth rate of osteoblast cells. [24] Thus, it can be concluded that the proposed composite coating layer could be a good alternative for bio implant coating applications.

So far, various techniques such as sol–gel, hydrothermal and microwave hydrothermal have been employed to synthesise HA/TiO2 nanocomposites. As a drawback, all these techniques require high-temperature heat treatment to obtain crystalline powder. In all these techniques, heat treatment is the only step that increased the expense and the other steps are simple and cheap to be prepared.

In contrast, in situ precipitation technique can produce HA/TiO2 crystalline nanocomposites through low-cost and low-temperature (room temperature) procedures. In this study, HA/TiO2 nanocomposite was synthesised through in situ precipitation technique from HA and titania precursors. In this novel technique, HA-TiO2 nanocomposite was synthesised by employing sol–gel and rapid breakdown adonisation (RBA) simultaneously. Morphology and microstructure of hydroxyapatite-grafted titanium nanotube composite (HA-TNT) were evaluated by scanning electron microscope (SEM) and transmission electron microscope (TEM). X-ray diffraction (XRD) was also utilised to characterise the composition and structure of the samples.

2. Materials and methods

Titanium sheet (5 cm × 5 cm), platinum foil, CaCl₂, Na₃PO₄ and ammonium hydroxide were purchased from Sigma Aldrich (Malaysia). Two precursors with 0.41 and 0.25 M concentration of CaCl₂ and Na₃PO₄ were prepared using distillated water. The pH of Na₃PO₄ solution was adjusted to 4.3 using NaOH. The CaCl₂ precursor was added to the Na₃PO₄ solution at the rate of 5 mm/min while differential potential of 20 V was applied to cathode (platinum foil) and anode (titanium sheet) with the distance of 2 cm. During one hour of RBA process, the solution was stirred at the rate of 450 rpm. This process was conducted for less than 1 hour until the initial foil was completely transformed into TiO₂ nanotubes powder to produce HA-TNT. Then, several centrifugal washing processes (10,000 rpm) were conducted in order to remove the Na and Cl residues which are soluble in the water solution. Finally, the composite was dried for 24 hours at 60 °C in an oven. In order to evaluate and compare the hardness of HA-TiO2 nanotubes composite, the pellets
of the composite and pure HA were prepared as control sample. Based on ASTM E92-82, a Vickers micro-hardness machine (SHIMADZU HMV 2T) was used to investigate the hardness of pellets. A diamond indenter with a standard geometry was indented under the load of 0.3 kgf into the pellet for 5 seconds. After the removal of the indenter, the contact area of the resulting indent was calculated (number of reputation is three) based on the following equation:

\[
HV = 1.854 \frac{F}{d^2}
\]

where \( F \) is load (kg force) and \( d \) is the total length of the diagonal indentation.

**Figure 1** FESEM image of synthesised HA-TNT composite.

**Figure 2.** TEM image of synthesised HA-TNT composite.
3. Results and discussions

Figure 1 shows the morphology of HA-TNT composite. The overall evaluation of the morphological feature indicates a uniform distribution of TiO$_2$ nanotubes in the HA matrix without agglomeration. As can be seen in Figure 1, TiO$_2$ of nanotubes structure are well synthesised and consequently well dispersed in the HA matrix. Moreover, TEM characterisation was conducted to confirm the structure of the synthesised TiO$_2$. In Figure 2, TEM image manifests that the HA-TNT composite consists of TNT with the diameter of approximately 10 nm. As shown in Figure 3, energy dispersive spectroscopy (EDS) analysis illustrated the presence of O (60.54 at. %), Ti (14.55 at. %), Ca (15.25 at. %) and p (9.65 at. %) with the Ca/P ratio of 1.58.

Figure 2 illustrates that the nanotube structures of TiO$_2$ have been surrounded by HA particles. This may be due to the in situ formation of HA when TNT was synthesised through RBA technique. The homogeneity of TNT into the HA matrix also can be inferred through the one-step procedure of the novel method used in this study.

Figure 4 shows the XRD pattern of HA-TNT composite recorded in the 2$\theta$ range of 20–70. The peak indexed at 2$\theta$ = 27, 57 and 65 is attributed to Rutile phase of TiO$_2$ nanotubes and the peaks at 2$\theta$ = 27, 57 and 65 corresponded to the Anatas phase of TiO$_2$ nanotubes. That 2$\theta$ = 23, 25, 28, 31, 32, 33, 35.5, 47, 49.5, 51, 52, 53, 54 and 56 confirms the formation of HA crystalline in the synthesised composite. This indicates that the calcium phosphate paste was formed in crystalline phase (HA). Moreover, it has been reported in several studies that HA is stable in simulated body fluid and is also desirable for bioapplications. [24] Furthermore, EDS spectrum in Figure 3 illustrates that the ratio of Ca/P is around 1.58 which confirms the theoretical value of HA in its chemical formula (Ca$_{10}$ (PO$_4$)$_6$(OH)$_2$). [25]

![Figure 3. The EDS spectrum and elemental analysis of synthesised HA-TNT composite.](image1)

![Figure 4. XRD pattern of synthesised HA-TNT composite.](image2)
The hardness of HA-TiO$_2$ nanotubes composite and pure HA was measured using Vickers micro-hardness device (Figure 5). The average hardness of the pellets (number of reputation is three) were 2.01 and 3.11 Gpa, respectively. The results revealed that there was an increase of 54% in the hardness of the composite pellet as compared to the pure HA pellet. Thus, it can be concluded that the incorporation of TiO$_2$ nanotubes into the matrix of HA possibly increased the micro-hardness due to the nature of TiO$_2$ in nano-scales. This result is in close agreement with the addition of TiO$_2$ nanoparticles in the matrix of HA reported by Que et al. and Okta et al. [26,27]

Due to the excellent mechanical and electrochemical properties of TiO$_2$ nanotubes, HA-TNT composite possibly has higher mechanical properties and corrosion resistance compared to the pure HA. Therefore, this composite can be considered an alternative bio-composite to the current HA composite coating layer on the metallic implant. This is of great consideration particularly in load-bearing applications where high mechanical properties of coated layer are required. In addition, the ion release from the coated implant can be reduced due to the fact that HA-TNT composite may have less porosity compared to the pure HA.

4. Conclusion

In this study, we attempted to develop a novel method of synthesising HA-TNT composite by combining sol–gel and RBA in a novel solution consisting NaCl and N$_3$PO$_4$, field emission scanning electron microscopy (FESEM) and TEM images from the synthesised composite illustrate the nanostructure of the composite with the TiO$_2$ nanotube diameter of approximately 10 nm. XRD and EDS analyses confirm the formation of HA crystalline, Anatas and Rutile phases. This novel one-step synthesis method is fast and economical and can be applied for fabrication of HA-TNT composites. Extraordinary mechanical and electrochemical properties of TiO$_2$ nanotubes make this composite an alternative candidate to the current HA for the coating layer of metallic implant in biomedical applications. Therefore, it can be recommended that this proposed in situ approach for synthesising the HA-TNT can be combined with the current commercial plasma spraying or electrospinning techniques which can provide simultaneous spray of HA and TNT on the implant surface and offer concurrent fabrication of HA-TNT scaffold with higher mechanical and electrochemical properties.

Figure 5. Hardness of HA-TiO$_2$ nanotubes composite and pure HA.
Acknowledgments

The authors are grateful to Materials Labs and also technicians of Physics Department at UTM for their help and support during performing part of this work. Moreover, the authors are thankful to Ministry of Higher Education (MOHE) for FRGS research funding (application ID: 118334) and relevant visiting researcher support. In addition, there are minor support from other funds to this study as follow; the Ministry of Science, Technology, and Innovation (MOSTI) for research funding vote 4S123, and UTM Research Management Centre (RMC) for PAS grants vote [grant number Q. J130000.2709.01K40] and [grant number Q. J130000.2709.01K41], and GUP grants vote [grant number Q.J130000.2609.11J04] and [grant number Q.J130000.2609.10J8]. The authors also would like to thank supporting members of above mentioned funds on their contribution.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors are thankful to Ministry of Higher Education (MOHE) for FRGS research funding (application ID: 118334) and relevant visiting researcher support. In addition, there are minor support from other funds to this study as follow; the Ministry of Science, Technology, and Innovation (MOSTI) for research funding vote 4S123, and UTM Research Management Centre (RMC) for PAS grants vote [grant number Q.J130000.2709.01K40] and [grant number Q. J130000.2709.01K41], and GUP grants vote [grant number Q.J130000.2609.11J04] and [grant number Q.J130000.2609.10J8].

ORCID

Arezou Shafaghat http://orcid.org/0000-0002-6439-936X
Ali Keyvanfar http://orcid.org/0000-0003-0059-274X

References

[1] Bezzi G, Celotti G, Landi E, et al. A novel sol—gel technique for hydroxyapatite preparation. Mater Chem Phys. 2003;78:816—824.
[2] Jafari S, Taheri MM, Idris J. Thick hydroxyapatite coating on Ti-6Al-4V through sol—gel method. Adv Mater Res Trans Tech Publ. 2012;341:48—52.
[3] Jafari S, Taheri MM, Idris J. Bioactive coating on stainless steel 316 L through sol-gel method. Adv Mater Res Trans Tech Publ. 2012;383:3944—3948.
[4] Assadian M, Shirdar MR, Idris MH, et al. Optimisation of electrophoretic deposition parameters in coating of metallic substrate by hydroxyapatite using response surface methodology. Arab J Sci Eng. 2015;40:923—933.
[5] Shirdar MR, Izman S, Taheri MM, et al. Effect of post-treatment techniques on corrosion and wettability of hydroxyapatite-coated Co—Cr—Mo alloy. Arab J Sci Eng 2015;40:1197—1203.
[6] Taheri MM, Abdul Kadir MR, Shokuhfar T, et al. Fluoridated hydroxyapatite nanorods as novel fillers for improving mechanical properties of dental composite: synthesis and application. Mater Des. 2015;82:119—125.
[7] Pushpakanth S, Srinivasan B, Sreedhar B, et al. An in situ approach to prepare nanorods of titania—hydroxyapatite (TiO₂—HAp) nanocomposite by microwave hydrothermal technique. Mater Chem Phys. 2008;107:492—498.
[8] Taheri MM, Abdul Kadir MR, Shokuhfar T, et al. Surfactant-assisted hydrothermal synthesis of fluoridated hydroxyapatite nanorods. Ceram Int. 2015;41:9867–9872.

[9] Abdel-Aal EA, El-Midany AA, El-Shall H. Mechanochemical—hydrothermal preparation of nano-crystallite hydroxyapatite using statistical design. Mater Chem Phys. 2008;112:202–207.

[10] Salimi MN, Bridson RH, Grover LM, et al. Effect of processing conditions on the formation of hydroxyapatite nanoparticles. Powder Technol. 2012;218:109–118.

[11] Eshtiaq-Hosseini H, Housaindokht MR, Chahkandi M. Effects of parameters of sol—gel process on the phase evolution of sol—gel-derived hydroxyapatite. Mater Chem Phys. 2007;106:310–316.

[12] Kheimehsari H, Izman S, Shirdar MR. Effects of HA-coating on the surface morphology and corrosion behavior of a Co-Cr-based implant in different conditions. J Mater Eng Perform. 2015;24:2294–2302.

[13] Li P, de Groot K. Better bioactive ceramics through sol—gel process. J Sol—Gel Sci Technol. 1994; 2:797–801.

[14] Bahrami M, Fathi MH, Ahmadian M. The effect of nanobioceramic reinforcement on mechanical and biological properties of Co-base alloy/hydroxyapatite nanocomposite. Mater Sci Eng C, Elsevier B.V. 2015;48:572–578.

[15] Mohseni E, Zalnezhad E, Bushroa AR. Comparative investigation on the adhesion of hydroxyapatite coating on Ti—6Al—4V implant: a review paper. Int J Adhes Adhes. 2014;8:238–257.

[16] Sung Y-M, Shin Y-K, Ryu J-J. Preparation of hydroxyapatite/zirconia bioceramic nanocomposites for orthopaedic and dental prosthesis applications. Nanotechnology 2007;18:065602.

[17] Quan R, Yang D, Wu X, et al. In vitro and in vivo biocompatibility of graded hydroxyapatite-zirconia composite bioceramic. J Mater Sci Mater Med. 2008;19:183–187.

[18] Ramires PA, Cosentino F, Milella E, et al. In vitro response of primary rat osteoblasts to titania/hydroxyapatite coatings compared with transformed human osteoblast-like cells. J Mater Sci Mater Med. 2002;13:797–801.

[19] Bellucci D, Sola A, Anesi A, et al. Bioactive glass/hydroxyapatite composites: mechanical properties and biological evaluation. Mater. Sci. Eng. C. Mater. Biol. Appl. 2015;51:196–205.

[20] Anmin H, Tong L, Ming L, et al. Preparation of nanocrystals hydroxyapatite/TiO2 compound by hydrothermal treatment. Appl Catal B Environ. 2006;63:41–44.

[21] Xu W, Hu W, Li M, et al. Sol—gel derived hydroxyapatite/titania biocoatings on titanium substrate. Mater Lett. 2006;60:1575–1578.

[22] Xiao XF, Liu RF, Zheng YZ. Characterization of hydroxyapatite/titania composite coatings codeposited by a hydrothermal—electrochemical method on titanium. Surf Coat Technol. 2006;200:4406–4413.

[23] Shokuhfar T, Sinha-Ray S, Sukotjo C, et al. Intercalation of anti-inflammatory drug molecules within TiO2 nanotubes. RSC Adv. Royal Soc Chem 2013;3:17380–17386.

[24] Oh S, Daraio C, Chen L-H, et al. Significantly accelerated osteoblast cell growth on aligned TiO2 nanotubes. J Biomed Mater Res A. 2006;78:97–103.

[25] Kuriakose TA, Kalkura SN, Palanichamy M, et al. Synthesis of stoichiometric nano crystalline hydroxyapatite by ethanol-based sol—gel technique at low temperature. J Cryst Growth. 2004;263:517–523.

[26] Oktar FN. Hydroxyapatite—TiO2 composites. Mater Lett. 2006;60:2207–2210.

[27] Que W, Khor KA, Xu JL, et al. Hydroxyapatite/titania nanocomposites derived by combining high-energy ball milling with spark plasma sintering processes. J Eur Ceram Soc. 2008;28:3083–3090.