Synthesis and application of titanium dioxide nanotube and composites

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SYNTHESIS AND APPLICATION OF
TITANIUM DIOXIDE
NANOTUBE AND COMPOSITES

LIANG KUN

SCHOOL OF ELECTRICAL AND ELECTRONIC
ENGINEERING

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**SYNTHESIS AND APPLICATION OF
TITANIUM DIOXIDE
NANOTUBE AND COMPOSITES**

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To my beloved parents, my husband

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Summary

As a multifunctional oxide semiconductor, titanium dioxide has attracted substantial interest for a wide range of applications, including photocatalysis, photovoltaics, electrochromics, biochemical sensing and drug delivery. On the other hand, enormous effort has been devoted to nanoscience and nanotechnologies. The increased surface area and surface-to-volume ratio benefits many devices based on nanomaterials. Various nanostructures of titanium dioxide have been investigated including nanowires, nanoparticles, nanofibres and nanotubes. This thesis is dedicated to the fabrication and application of titanium dioxide nanotubes and its composites.

Titanium dioxide nanotube structures have been successfully fabricated by anodization method. The nanotube aspects, including length, diameter, outlooks, can be tailored by varying experimental parameters such as electrolyte, anodic voltage, experimental time and repeat usage of electrolyte. TiO_2 nanotubes with diameter up to 700 nm have been fabricated successfully for the first time by repeat use of electrolyte at high supplied potential, mainly due to the new established balance between oxidation and dissolution. Double-walled titania tubular structure is successfully fabricated by low temperature ($0^\circ\text{C} \pm 3^\circ\text{C}$) anodization followed with subsequent annealing. The low temperature provided tube with thick walls and low porosity. The heating process introduced the split to two concentric tubes, which was mainly because of the mechanical radical strains from the release of impurity atoms and volume shrinking. The experimental details and characterizations of the various titanium dioxide nanotubes are presented in Chapter 3.

As a popular implant material in bone graft, the surface of titanium is usually treated with bone morphogenetic proteins (BMPs) to enhance surgery effect. TiO₂ nanotubes are one of the most popular coatings. The influences on protein elution by different tube parameters including lengths diameters and phases are investigated and presented in Chapter 4. In general, Titania nanotubes provide super hydrophilic surface and more loading volume for proteins. Tube diameter and density has obvious effect on protein elution. Wider diameter and larger gaps allow ease of entering as well as eluting solvent from the nanotubes. The elution is most profoundly affected by tube length. Samples with longer tubes provide more loading sites and allow deeper penetration. The change of material phase doesn't affect surface wettabilities but does introduce some structure change which further provides minor influences on the overall elution.

Titanium dioxide is also well known as one of the most popular photocatalysts. The photocatalytic properties of various TiO₂ nanotube structures are investigated for methylene blue decomposition under both UV and white light irradiation. In Chapter 5, we shall report the study on photocatalytic properties of titania nanotubes. Overall, tubular structures provide much larger surface area hence excellent decomposition efficiency for MB. The fabricated double-walled TiO₂ nanotubes demonstrated excellent degradation efficiency as compared to single-walled structure prepared from same electrolyte. The double-walled structures have the capability to provide more reaction sites and excess oxygen absorption.

To further improve the photocatalytic property of titanium dioxide, varieties of composite materials were studied in the last period. Chapter 6 presents the fabrication of TiO_2 – C composites and their application on water purification. Various 3D titanium dioxide and carbon composites were successfully fabricated: TiO_2 nanotube with secondary CNT, CNT/CNT network with titania coating and graphite / graphene with titania coating. All the fabricated composites showed improvement on photocatalytic efficiencies especially under UV irradiation. Carbon materials could act as ideal places for physisorption of contaminants. Moreover, carbon can act as photosensitizer to absorb visible light and conduct photo-induced charges away to reduce the recombination rate.

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Common Abbreviations

3D	Three dimensional
BET	Brunauer-Emmett-Teller
BMP	Bone Morphogenetic Protein
BSA	Bovine serum albumin
BSC	Bio safety cabinet
CA	Contact angle
CB	Conduction band
CNT	Carbon nanotube
DC	Direct current
DEG	Diethylene glycol
DI	Deionized
DMSO	Dimethyl sulfoxide
EDX	Energy dispersive X-ray Spectroscopy
EG	Ethylene glycol
ELISA	Enzyme-linked immunosorbent assay
HA	Hydroxyapatite
IPA	Isopropyl alcohol

MB	Methylene blue
NT	Nanotube
P25	Titanium dioxide, anatase : rutile = 75 : 25
PBS	Phosphate buffered saline
PECVD	Plasma enhanced chemical vapor deposition
PGA	Polyglycolide
PLA	Polylactide
PLLA	Poly-L-lactide
PTFE	Polytetrafluoroethylene
SEM	Scanning Electron Microscope
TCVD	Thermal chemical vapor deposition
TEM	Transmission Electron Microscope
TiO ₂	Titanium Dioxide
UV	Ultraviolet
Vis	Visible

Chapter 1 Introduction

1.1 Background

Titanium dioxide is an oxide semiconductor, also known as titania. Besides amorphous, titanium dioxide appears in nature at three well-known phases: anatase, rutile and brookite [1]. Among the three phases, rutile is the most stable natural form while anatase phase is the one most explored for various applications. Titanium dioxide has been commercially used as pigments, paints and so on [2, 3]. Since the discovery of photocatalytic property of titanium dioxide by Fujishima and Honda [4, 5], other promising applications have been explored such as photocatalysis, photovoltaics, electrochromics and sensors [6-9]. Furthermore, titanium itself has been used as implant material for a long time [10]. Titanium dioxide also drew more attention in bio-related area, such as biosensor, implant coating and drug delivery, because of its good bio compatibility [11-13].

Not only the properties of titanium dioxide, but also the structures of titania were studied. Enormous effort has been devoted to nanoscience and nanotechnologies [14, 15]. As the size of material decreases, the surface area and surface-to-volume ratio increase [16]. This benefits many devices based on titanium dioxide, as certain applications such as photocatalysis strongly depend on surface area. Various nanostructures of titanium dioxide have been investigated including nanoporous film, nanoparticles, nanofibres, nanowires and nanotubes [17-21]. There are numbers of techniques that have been

developed for preparing titanium dioxide nanostructures, such as hydrothermal, sol-gel, templating synthesis and electrochemical anodization [22-25].

Among the various nanostructures of titanium dioxide, nanotubes have drawn much interest because of its unique combination of shapes and functionalities. Nanotubular titania has been widely studied in surface area dependent applications including photocatalysis, sensors, and dye-sensitized solar cells. Besides, titanium dioxide nanotubes have also presented remarkable properties for application in biosensors, tissue engineering and drug deliveries [26-30].

Nanotube structures have the advantages on surface area extension and surface-to-volume ratio increase which could benefit many devices depending on surface area [16]. In current state-of-art nanotube structures, TiO_2 nanotube and CNT are the most popular ones. In fabrication, both nanotube structures could have wide diameter and length range. In applications, both nanotube structures have been used in various applications in environmental and bio areas. Both CNT and TiO_2 nanotube are widely studied in biosensors, tissue engineering and drug deliveries [26-33]. TiO_2 has better biocompatibility and Ti has long history of being used in medical application, such as dental implant, hip joint prostheses and stents, because of its superior bulk and surface properties compared to other materials [34, 35]. On the other hand, CNT's biocompatibility is not as good as TiO_2 . Even CNT has also been used in bio areas; there are still doubts about its toxicity [36-38]. Particularly for drug delivery, the hydrophilic surface of TiO_2 provides better biocompatibility but slow release rate. On the other hand, CNT has hydrophobic surface which

promotes the release of drug but has less biocompatibility. Furthermore, both nanotube structures can be used in environmental application. Titanium dioxide has photocatalytic property and is one of the most popular photocatalysts. Its coatings have been widely used in various applications including sterilization, air purification, water purification, defogger, self-cleaning and hydrogen generations [5, 39-46]. As catalyst, TiO_2 can be used many times and has long term stability on its photocatalytic property. CNTs are not photocatalyst but can also be used in environmental pollution management, mainly act as adsorbent due to its good physisorption properties [47-49]. As adsorbent, CNTs has saturation point on its adsorption ability.

This work focuses on the fabrication of titanium dioxide and its composites with carbon materials. Applications in photocatalysis and protein deliveries are both investigated with the fabricated nanotubular structures. In this section, brief introduction to fabrication of titanium dioxide by anodization technique will be firstly given. A description on bone morphogenetic protein-2 and its delivery systems is presented next, followed by an introduction to the photocatalytic properties of titanium dioxide.

1.1.1 Fabrication of TiO_2 nanotubes

For TiO_2 nanotube fabrication, three popular techniques have been studied in recent years: template-assisted method, hydrothermal treatment and electrochemical anodization. With template-assisted method, dimensions (such as diameter and length) of TiO_2 nanotubes can be controlled by the dimensions of template (eg. CNT template, anodized alumina oxide template) [50]. Hence, the range of tube diameters and lengths of the fabricated TiO_2 nanotube are

usually limited by the dimensions of the template. TiO_2 nanotubes are obtained after removal of templates which may destroy the tubular structure and result in contamination [50, 51]. With hydrothermal treatment, TiO_2 nanotube can be fabricated in large amount but usually have random alignment and non-uniform diameters [51, 52]. The length of nanotubes produced is also random. Furthermore, hydrothermal treatment may produce other nanomaterials such as nanowires, nanofibers and nanorods during the fabrication process. Electrochemical anodization could produce TiO_2 nanotubes with not only oriented alignment but also relatively uniform diameters [3]. The dimension of nanotubes could be easily controlled by varying experimental parameters such as voltage, electrolyte, anodization time and so on [51, 53, 54]. The first self organized TiO_2 nanostructure was grown by anodization of Ti in fluoride contained electrolyte in 1999 [55]. Since then, anodization has become one of the most effective and simple methods in fabrication of nanotube arrays.

Metal is used as anode during the electrochemical process to produce oxide layers, that's why the electrochemical technique is also called anodization. The main factor for nanotube formation is the fluoride ions in electrolyte. Figure 1.1 (a) [53] demonstrates the situation for titanium anodization in electrolyte with and without fluoride ions. If the electrolyte contains no fluoride ions, a compact oxide layer or sometimes a disordered nanoporous layer could be formed. On the other hand, with fluoride ions in electrolyte, self-ordered nanoporous or nanotube layer would be fabricated.

The mechanism of nanotube formation is demonstrated in Figure 1.1 (b) [53]. There are two competition processes: oxidation and dissolution (or etching).

Metal (titanium) surface is oxidized through reaction (1.1). Oxidized metal species react with O^{2-} from water to form an oxide layer. This process describes the formation of compact layer (Figure 1.1(a)) [53, 56]. In the presence of fluoride ions, water-soluble $[\text{TiF}_6]^{2-}$ can be formed according to reaction (1.2) and Ti^{4+} ions can be solvated to $[\text{TiF}_6]^{2-}$ (reaction 1.3) to avoid precipitation [56]. Surface topological fluctuation initiates local field distribution difference and the initial pore is formed by field-enhanced dissolution. The competition between oxidation and dissolution exists throughout the whole fabrication process. A more detailed experimental setup and mechanism discussion will be presented in Chapter 2.

Figure 1.1 (a) Anodization of titanium in electrolyte without and with fluoride ions. (b)

Schematic mechanism for nanotube growth.

In anodization, the morphologies of titanium dioxide nanotube, such as diameter, length and density, could be tailored by experimental variables including experimental duration, anodization voltages and electrolyte composition [53, 56, 57]. In general, tube diameter is linearly related to the applied voltage if other experimental parameters are kept the same [56, 58]. This linear relation could be presented by $R = f_{growth} \cdot U$, where R is the radius, U is the applied potential and f_{growth} is the defined growth factor. The value of growth factor differs for different metals and for Ti / TiO₂ the value is 2.5 nm / V [59, 60]. However, tube diameter cannot increase infinitely. There is usually a voltage range for certain electrolyte. Too low voltage cannot initiate tube growth due to the small vertical electrical field and too high voltage clogs tubes because of fast dissolution rate [61]. If all chemical parameters are kept constant, length of nanotube is usually controlled by the anodization duration [57, 58]. However, when the experiment is held long enough, the competition between oxidation and dissolution would reach a steady-state so that there would be no increase in length [61, 62].

Besides time and voltage, electrolyte is an essential factor to tailor nanotube geometries. Electrolyte pH, concentration, composition and viscosity could influence the growth of nanotube largely [54, 56, 58, 61, 63]. Depending on different electrolytes used, the anodization technique could be classified into four different synthesis generations [64]. The detailed information of the four generations is shown in Table 1.1.

Table 1.1 Synthesis generations of TiO₂ nanotube.

No.	Generation	Example Electrolyte	Voltage	Length	Diameter
1	Aqueous electrolyte	HF based aqueous solution	0 - 20V	~ 500 nm	15 - 30 nm
2	Buffered electrolyte	NaF, KF solutions	10 - 25 V	2μm – 5 μm	40 - 110 nm
3	Organic electrolyte	F ⁻ Containing organic solvent eg. DMSO, DEG, EG with HF or NH ₄ F mixture	30 – 70 V (can reach 100+ V for NH ₄ F)	× 10 μm – × 100 μm	100 – 350 nm
4	Non-F electrolyte	No F ⁻ solutions. eg. HCl, H ₂ O ₂ mixture	10-23 V	< 1μm	~ 15 nm

The first generation of TiO₂ nanotube fabrication used aqueous HF solution and the anodization voltages applied was usually below 20 V [65-67]. The fabricated nanotube structures have short length up to 500 nm and small diameter commonly 15 – 30 nm. An example is shown in Figure 1.2(a). The rapid dissolution rate restricted both tube diameter and length in the first generation. Electrolyte PH was adjusted by using KF or NaF with a buffer solution in the second synthesis generation [68, 69]. The hydrolysis rate increased with increasing PH; hence the chemical dissolution rate was reduced resulting in longer tubes. Anodization potential of 10 V – 25 V could be used to induce tubes with lengths up to 5 μm and diameters up to 110 nm. An example of nanotube fabricated from second generation is shown in Figure 1.2(b). In aqueous solution, the fast dissolution rate is mainly due to the water content. To reduce water content, the third generation of fabrication used organic electrolytes such as ethylene glycol (EG), diethylene glycol (DEG) and dimethyl sulfoxide (DMSO) [70-72]. The fluoride provider was usually hydrogen fluoride or ammonia fluoride. Tube length is significantly enlarged

with the reduced etching rate, which is mainly induced by reduction on water content. The tube length could reach tens or hundreds of micrometers. Furthermore, the fabricated tube diameters are also obviously enlarged. Anodization voltage over one hundred volts could be applied and tube diameter could reach up to 350 nm. An example is shown in Figure 1.2(c). The fourth fabrication synthesis was commonly regarded as anodization with non-fluoride based electrolytes. The electrolyte could include mixtures of HCl, H₂O₂ and mixtures of oxalic acid, sulfuric acid with NH₄Cl [73-76]. If aqueous solvent is used, the fabricated nanotubes are quite alike with that from the first generation: nanotubes were short and thin. If the solvent is changed to organic, length could reach to several microns. The applied voltages used ranged from several to 25V.

Figure 1.2 SEM images of TiO₂ nanotube example from anodization synthesis of (a) first generation, (b) second generation and (c) third generation. Figures are from reference [45].

Noticed among the four generations, the organic electrolyte could provide titanium nanotubes with widest range of diameter and length. This is also the type of electrolyte studied in this work.

1.1.2 BMP-2 and its delivery system

The osteoinduction phenomenon was first described in 1965 because of the presence of a protein in bone matrix; this protein was named bone morphogenetic protein (BMP) [77, 78]. BMPs are usually demineralized from bone matrix. They belong to the transforming growth factor which is important for tissue repair in postnatal life [79, 80]. The discovery of BMP is a major step in development of advanced method in bone surgery. There are seventeen BMPs that have been isolated and characterized to date, named from BMP-2 to BMP-18 [81-83]. Among them, BMP-2 and BMP-7 have been approved by the US Food and Drug Administration (FDA) for clinical use [84, 85]. BMP-2 is fully approved to be used in surgeries such as anterior lumbar interbody fusion and acute open tibia fracture [84]. In this work, the protein used is BMP-2. In the following thesis, the ‘BMP’ or ‘BMPs’ refer to BMP-2 if there is no special explanation.

BMPs could induce bone formation by triggering mesenchymal stem cells to differentiate into osteoblasts or bone cells, hence influence bone and cartilage formation [84, 85]. After administration, chondroblast differentiation was observed on day 5 and the mark of osteoblasts was most active on days 10 – 12 [85]. This suggests that BMPs take about one week’s time to stabilize. Furthermore, it was also found that the osteoinductivity of BMPs was dose dependent [86]. Threshold concentrations need to be achieved to get effective osteoinductivity. In human, a total dose of 4.2-12mg of BMP-2 at 1.5mg/ml is recommended for anterior inter-body fusion [86]. The amounts needed for different surgeries are different. After administration, BMP is immediately

subjected to the presence of fluid, protein competition, enzymatic activity, temperature, pH variation, pressure and salt concentration [87, 88]. Some of the BMPs can be ‘killed’ and lose their bioactivity. This implies that a higher dose needs to be loaded. It is said that BMPs are used in current clinical applications at concentrations that are ten to 1000-fold higher than those of native BMPs [84]. Furthermore, all these factors could lead to denatured or uncontrolled bone growth. Not only the cost for surgeries is increased, the success of clinical BMP therapies is also limited. This raises the importance of developing effective delivery system or carriers that are biocompatible and can maintain BMPs’ bioactivity.

Carriers or delivery systems not only help to maintain the concentration of BMPs at the site but also prevent protein diffusion. BMPs are soluble and the clearance is very quick if delivered in a buffer directly. Only 5 % remains at the site. However, the retention range is increased from 15 % to 55 % if combined with carriers (gelatin foam or collagen) [84, 89]. “Diamond concept” [90] was raised to summarize requirements for a carrier. Stability should be established among the defect site, osteogenic cells, osteoinductive growth factors and the carriers. The carrier should be biocompatible so that it is friendly to cells. At the same time, carriers should be able to enhance attachment of BMPs and reduce protein loss.

There are mainly four types of delivery systems classified by materials as shown in Table 1.2 [91, 92]. Natural polymers, including collagen, hyaluronic acid, fibrin and other naturally derived polysaccharide, have the advantages of being biocompatible and affinity with BMPs [93-95]. Currently collagen-based

formulation is the only specific carriers approved by FDA for limited clinical use [96]. The major disadvantages include processing difficulties, sterilization difficulties, possible disease transmission and immunogenicity [97]. Examples of inorganic materials are ceramic such as hydroxyapatite (HA) and non-ceramics such as calcium phosphate [98-101]. This type of material has chemical nature identical to that of bone and promotes bone formation directly on their surfaces. The ceramic and coatings are brittle and difficult to mold with decreased mechanical tensile and shear properties [91]. Synthetic polymers like PLA and PGA are studied for BMP delivery too [94]. Degradation of polymer occurs by hydrolysis, enzymatic pathways; this could induce break-down products and chronic inflammation which could cause local PH decrease and detrimental to the stability of BMPs [91, 92, 97, 102]. Composites of the above materials could also be used for protein delivery, such as calcium phosphate on collagen sponge [103] and PLLA-HA-collagen scaffold [104, 105]. Although the manufacturing process is complex, composite have shown some optimized benefits.

Table 1.2 Major classes of carrier materials and comparison.

Carrier	Adv.	Disadv.	Types
Natural polymer	Biocompatible, biodegradable, natural affinity for BMPs	Immunogenicity, sterilization difficulties, pathogen transmission	Collagen (gel, nanofibres, scaffolds and films), fibrin glue, chitosan, heparin, hyaluronic acid
Inorganic material	Similar to bone degradable / non-degradable, osteoconductive, self setting ability in vivo, affinity	Brittle, difficult mold, some calcium phosphate cements are exothermic	Hydroxyapatite, tri-calcium phosphates, metals, ceramics and calcium sulfate
Synthetic polymers	Excellent chemical and mechanical properties, easy process and sterilize, flexible to tailor and reproducible	Inflammatory response, localized pH drop due to by-products acidity, limited biological function	Polymers (PLA, PGA), copolymers (PLGA)
Composites	Combination of different material	Complex manufacturing	Collagen-HA, Titanium-PLLA

The above mentioned materials have been made into different geometries and configurations such membranes, blocks, matrices, microparticles, hydrogels and foams. Some structures used are porous membranes, spheres, fibers and particles. Depending on carrier materials and structures, BMPs could be immobilized by non-covalent or covalent bindings [106]. Non-covalent bindings are normally physical trap or adsorption of BMPs into the carrier matrix. Covalent bindings are usually chemical binding through some functional groups and may affect the bioactivity of protein. The immobilized proteins could be released through diffusion control, chemical reaction control, solvent control or a combination of the above [86, 107]. No matter which type of bindings or elution, a good carrier of BMPs should give high loading capacity, maintain protein bioactivity and minimize irreversible protein binding.

As mentioned earlier, threshold concentration needs to be achieved in order to get effective bone repair and regeneration. In addition, only after several days the chondroblast differentiation was observed since the administration of BMPs [97]. So a carrier is not only required to delivery protein to the targeted defect sites, but also should provide enough capacity and long retention. Most of current delivery systems could achieve targeted delivery. Many observed bone growths are reported with various carriers [92]. However, it was reported by Haidar in 2009 that “no single clinically-effective delivery system for rhBMP-2, rhBMP-7 or any other bone growth factors has been developed to date” [91]. Even though there is improved bone growth effect observed in many vivo reports, the release of BMPs is still much faster than demanded. Some types of carriers can maintain the protein’s bioactivity very well and have small amount of irreversible protein, but the initial release is too high [108]. If the initial loaded dose is too high and concentrates out of the implanted position, unnatural bone growth may happen at undesired sites. While other types of carriers can give long retention time but couldn’t release full loaded protein [100]. To get effective bone regeneration, a higher initial BMP loading may be required which implies higher surgery cost and increases risk of complication. A more effective carrier or delivery system is required to provide controllable release of BMPs.

Nanomaterial has drawn much attention in bio-related applications like regenerative medicine. Nanotechnology is an excellent tool to produce material structures that mimic the biological ones and holds the promise of providing efficient delivery systems [109]. Nanomaterials like nanoparticles, nanofibres,

and nanocarriers have been used for drug delivery, tissue scaffold and biological sensor [110]. It is well known that the physicochemical properties of the biomaterial surface like surface energy, surface charge or chemical composition affect the cell response. One big advantage of nanomaterial is that the surface area is enlarged greatly compared to micromaterial. As mentioned in the previous delivery system section, the nano-fibrous and nano-particles have demonstrated superiority in terms of longer residencies in general circulation, consequently extending the bioactivity of the entrapped molecule compared to microscale material [111].

Titanium has been used in medical applications, such as dental implant, hip joint prostheses and stents, because of its superior bulk and surface properties compared to other materials [34, 35]. It is actually the naturally formed thin oxide layer on the surface that has direct interaction with living environment. Titanium dioxide has low inherent toxicity, low solubility in water, small reactivity with biomolecules and advantageous peroxide chemistry with apparent anti-inflammatory action [112]. Furthermore, as mentioned, titanium dioxide has various nanostructures including nano-porous films, nano-particle, nano-fibers, nano-wires and nanotubes. Especially for nanotubes, tube configurations could be easily altered by different experimental parameters, which could provide various surface morphologies. It has been quite acknowledged that nanoscale topographies of biomaterial surface can influence cellular interactions such as osteoblast adhesion and further biomineralization [113, 114]. The formation of nanostructures on the surface usually enhances human cell growth mainly due to the increased surface area and adhesion.

Studies have indicated that cells prefer nanostructured titanium dioxide with a more hydrophilic character and higher electrochemical stability [115, 116].

The combination of BMP and TiO₂ nanotubes on the surface of implant has been studied for bone repair. Loosening of an implant from the host bone due to poor osteo-integration has attributed to implant failure. Porous titanium and anodized surface with BMP loading showed a significant reinforcement and contribution to a better joint function, because of the lower release velocity and sustained bioactivity [117-119]. Figure 1.3 shows that osteoblast adhesion is increased on anodized Ti surface than on unanodized surface [119]. With immobilized BMP-2 knuckle peptide loaded on anodized tubular Ti surface, osteoblast adhesion is even further improved. This result not only shows BMP is a promising solution for bone repair but further proves that TiO₂ nanostructure can be a promising configuration for BMP sustained delivery [11, 119-121].

Figure 1.3 Increased osteoblast adhesions on anodized Ti surface. Right top: anodized TiO₂ surface; bottom: tubular TiO₂ with BMP-2 loading.

1.1.3 Photocatalysis

In 1972, the photo-electrochemical decomposition of water was found on titanium dioxide surface under UV irradiation and applied bias [4, 5]. This splitting mechanism is induced by photocatalytic process. After the discovery, photocatalysis has been widely investigated on degradation of various organic compounds in water [122-125]. Among all the photocatalysts, titanium dioxide is the most popular one because of its efficient, chemical and photo-chemical stabilities while being of easy-prepare and low-cost [126]. Titanium dioxide and its coatings have been widely used in various applications including sterilization, air purification, water purification, defogger, self-cleaning and hydrogen generations [5, 39-46].

Photocatalysis can be generally considered as catalysis of photochemical reactions at the surface of a semiconductor, here is titanium dioxide [127]. Light (photons) and catalyst are necessary for photocatalytic activity. There are usually two reactions occurring at the same time: oxidation from holes and reduction from electrons photogenerated. The initial process for photocatalysis is the generation or excitation of electron and hole pairs. Figure 1.4 shows schematic of the electron-hole pair excitation [123, 128]. Electrons in valence band would absorb photons, with energies equal to or larger than the band gap of titanium dioxide, and excite to conduction band, creating electron and hole pairs. Majority of pairs would recombine very quickly at the surface (path A) or inside the volume (path B). Holes migrated to surface have high tendency and power for oxidation. They can take the electron from hydroxide ion and produce hydroxyl radicals (path D), which could further promote oxidation of

harmful substances. Electron can be donated to reduce an electron acceptor such as oxygen in solution (path C), followed by radical chain reactions [129]. The organic substances would eventually decompose under the photocatalytic activities.

Figure 1.4 Schematic of events in a photocatalyst after UV irradiation.

Only anatase phase of titanium dioxide has photocatalytic property and the bandgap is 3.2 eV, which means only light with wavelengths less than 390 nm ($h\nu > 3.2\text{eV} \rightarrow \lambda < \frac{1240}{3.2}\text{nm}$) could be absorbed and used for electron excitation [130]. This limits the use of photons to UV range that amount to about 2.5 % of the solar spectrum reaching the earth's surface [131]. One good aspect about UV irradiation is that the TiO_2 surfaces become superhydrophilic [129]. The self-cleaning property of titanium dioxide coating is generated mostly from the superhydrophilic surface as the contaminants could be removed with the help of moisture. Other than UV, how to efficiently use visible light from solar energy is one of the challenges to improve photocatalytic efficiencies. Many efforts have been devoted to extend the response wavelength of titanium dioxide to visible light range, including doping with metal impurities, doping with non-metal atoms and coupling TiO_2 with

narrow band gap semiconductors [127, 132-139]. Non-metal atom doping, such as N [133, 134], S [137], C [138] and F [139], was reported the most promising method to shift the absorption response to visible range. The non-metal doping could either narrow the band gap or introduce midgap level, both of which can devote to the visible light absorption [127].

The other challenge to improve photocatalytic efficiencies is the high recombination rate of the photoinduced carriers. As mentioned earlier, there are deexcitations while the electron-hole pairs are being generated. Researchers have used laser impulse to study charge carrier kinetics and it was found that more than 90 % of the photo-generated electron-hole pairs recombined within 10 ns after laser pulse [140]. In order to improve photocatalytic efficiency, it is also important to enhance charge separation. Nobel metal coating on titanium dioxide surface could improve the efficiency. Surface coating by Pt and Au significantly improves the photocatalytic property [141, 142]. The metal particles act as electron sinks which enhance charge separation and reduce charge recombination rate [140]. Recently, it was found that combination with π structured materials, such as carbon nanotubes and graphene, could also improve photocatalytic efficiency [143-147]. The carbon material has good conductivity and can reduce the recombination rate as metal coating does.

Nanostructured titanium dioxide has attracted much attention. Many nanostructures were fabricated including sphere, nanorods, fibers and tubes [148-151]. One of the simplest ways to improve photocatalytic efficiency is to enlarge the specific surface areas [129]. With nanostructured titanium dioxide, the surface area could be enlarged significantly hence resulting in higher rate of

photocatalytic decomposition. As stated in earlier section, TiO₂ nanotubes with various morphologies could be fabricated easily by anodization. Nanotube structures provide superior decomposition of organic pollutants because of not only the larger surface area but also the facile diffusion of organic into the nanotubes. Furthermore, nanotubes with thin walls could reduce recombination of electron and hole pairs by photo-adsorption if the half wall thickness is less than the carrier diffusion length [129]. It has been also observed by some researchers that titanium dioxide nanotubes demonstrated better photocatalytic performance than P25 in a comparable way [152].

In all the extra materials introduced so far that can be composited with titanium dioxide, carbon deserves special attention. Not only carbon doping could extend the absorption response of titanium dioxide to visible light range, carbon material in contact with TiO₂ could also reduce charge recombination. So carbon could promote photocatalytic efficiency of titanium dioxide in both ways, which draws TiO₂-C composite much interest in photocatalysis. Various composites have been fabricated and studied. With carbon doped titanium dioxide, improved photo-decomposition under visible light ($\lambda > 420$ nm) was observed [153-155]. Composites of titanium dioxide and carbon (CNT and graphene) were also fabricated including CNT with TiO₂ coating, titania particles on CNT surfaces, graphene mixed with titania nanorods and so on [156-159]. In most fabrications, composites were usually prepared by mixing of commercially bought carbon and titanium dioxide with the help of chemicals. Besides electron sink, CNT may act as photosensitizer to extend TiO₂ photoactivity to visible light range. In addition, graphene and CNT can provide

extra dye absorption and band gap narrowing resulted from Ti-O-C bands[160]. CNTs are also popular in environmental pollution management, mainly act as adsorbent due to its good physisorption properties [47-49].

1.2 Motivation and Objectives

Titanium dioxide nanotubes can be fabricated from a one-step electrochemical anodization. The tube morphologies can be easily tailored by altering experimental factors such as anodization duration, applied voltage and electrolyte composition. Among all types of electrolytes, organic electrolyte can produce nanotubes with most variety in morphologies. There is usually a range limit for the applied voltage, as too high voltage clogs the formation of nanotubes because of the fast etching rate and too low voltage cannot initiate tube growth due to the small vertical electrical field. It is a challenge to find a way to fabricate nanotubes beyond the limit.

Titanium dioxide nanotubes can be used in many applications. One of the applications is in bone repair. Ti has proven itself to be a good implant material because of its good biocompatibility and mechanical properties. TiO₂ nanotube coatings have demonstrated enhanced cell adhesion and superiority in terms of longer residencies of trapped molecules. The combination of titanium dioxide and BMP-2 has shown improved osteoblast adhesion and enhanced bone repair effect. Even though it is proven that titanium nanotube can be a good carrier for BMP-2 elution, influences from different nanotube aspects (diameter, length, density) on protein delivery profiles are seldom reported.

The most essential and well-known property of titanium dioxide is its photocatalytic property. Titanium dioxide is one of the most popular photocatalysts in clean energy. It is essential to continuously improve the photocatalytic efficiency in order to extend its applications. One of the simplest ways is to enlarge the specific surface area. TiO_2 nanotubes, easily fabricated from anodization, demonstrate enhanced photoactivity because of its enlarged surface area. Detailed studies towards the photocatalytic efficiencies under both UV and visible irradiation are highly desirable for nanotubes with different morphologies. Titanium dioxide composited with carbon can also enhance its photocatalytic property not only by introducing electron sinks but also by acting as photo-sensitizer. Most of current composites prepared are films and powders from commercially bought carbon and titanium dioxide materials. It would be great advantage to enhance the photocatalytic activity of titanium dioxide by preparing 3D structured composite.

In attempting to take up the above challenges on fabrication and application of titanium dioxide nanotubes, the main objectives of this thesis are:

1. To fabricate titanium dioxide with different structures by electrochemical anodization in organic electrolytes; voltages beyond the limit will also be applied.
2. To investigate the influences on elution profiles of BMP-2 by titanium dioxide nanotubes carriers with various tube parameters.
3. To study the photocatalytic properties of titanium dioxide nanotubes with various tube morphologies under both UV and visible irradiation.

4. To explore the fabrication of 3D TiO₂-C composites and their enhancements on photocatalytic efficiency.

1.3 Major Contributions

The main contributions of this work are as follows:

1. The fabrication of various titanium dioxide nanotube structures by anodization has been achieved. Especially, the formation mechanism of nanotubes with larger diameter and double-walled structures were addressed.
2. Titania nanotubes have been utilized as carriers for bone morphogenetic protein – 2 deliveries. Controlled release of protein has been achieved by altering nanotube diameter, length and material phases.
3. Photocatalytic property of titanium dioxide has been enhanced by nanotube structure. The photoactivity is enhanced further with double-walled nanotube both under UV and visible irradiation.
4. Further enhancing of the photocatalytic property of titania has been achieved by titanium dioxide and carbon composites. Titanium dioxide nanotube and secondary CNT networks composite has been achieved by TCVD method. CNT/CNT network – titanium dioxide and graphite/graphene – titanium dioxide mixture were fabricated by solvothermal coating on carbon substrates.

1.4 Organization

Organization of this thesis is as follows:

Chapter 1 introduces basic background information on titanium dioxide nanotube fabrication, its application on protein delivery and photocatalysis, followed by the motivation and objectives of this thesis. The main contributions and organization of the thesis are also given in this section;

Chapter 2 illuminates the fabrication method of titanium dioxide nanotube, TCVD method, ELISA method, photocatalysis test and various characterization techniques adopted in this work;

Chapter 3 presents the fabrication and characterization of titanium dioxide nanotubes by anodization with organic electrolytes. The preparation of titania nanotube with large diameters and double-walled structures have also been investigated;

In Chapter 4, the investigation on BMP-2 delivery kinetics with various titanium dioxide nanotube structures are presented. The influences from different nanotube aspects such as diameter, length and phase are also studied;

Chapter 5 presents the study on photocatalytic properties of various titanium dioxide nanotube structures. Both UV and visible irradiation are used for the investigation of photoactivity. Double-walled nanotubes are also investigated for water purification;

Chapter 6 presents the fabrication and characterization of titania and carbon composites. TiO₂ nanotube – secondary CNT, CNT/CNT network – TiO₂ and

graphite/graphene – TiO₂ composites are prepared and studied for their enhanced photocatalytic properties.

Chapter 7 summarizes all the work that presented in this thesis and explores possible future research recommendations in related areas.

Chapter 2 Experimental and Methodology

In this chapter, most of the experimental and characterization methodologies are introduced and discussed. The experimental methods including anodization method, TCVD and ELISA are briefly discussed in the first part. The second part of the chapter describes basic principle and measurement capabilities of characterization tools such as SEM, Raman, UV-Vis spectrometer and so on. Depending upon the content, separate descriptions will be given for the techniques that not appeared in this part.

2.1 Experimental

2.1.1 Fabrication of TiO₂ nanotube structure by electrochemical anodization

Electrochemical anodization method or anodization for short is one of the simplest, cheapest and straight-forward approaches for fabrication of nanoporous structures. Anodization technique can lead to highly ordered, self-organized porous membranes or nanotubes for plenty of metals such as Al, Ti, W and so on [161-163]. As briefed in Chapter 1, TiO₂ nanotubes with diameters from 30-300 nm and lengths from several to tens of micrometers can be easily fabricated by this method. Anodization also has the advantage as a wet chemical process: it is easy and convenient for different shapes of substrates.

Anodization is a top-down process by chemical etching. The experimental set up is usually a two-electrode or three-electrode cell. A two-electrode cell is shown in Figure 2.1(a) [53]. The container used is usually a PTFE container to

prevent possible corrosion from the acidic or F^- contained electrolyte. A sheet of metal, in this thesis Ti ($2\text{ cm} \times 2\text{ cm}$), works as both anode and substrate. Cathode used is normally platinum wire or mesh. If it is a three-electrode cell, there will be an added reference or common electrode. A DC voltage supply is applied to anode and cathode.

Figure 2.1 Anodization experiment setup (a) and mechanism (b).

The actual fabrication process is a competition between two processes: oxidation (growing) and dissolution (etching), as displayed in Figure 2.1(b).

Metal (Ti) surface is oxidized through reaction (2.1). Oxidized metal species react with O^{2-} from water to form an oxide layer. Ti^{4+} will transport upwards to the electrolyte and O^{2-} will transport to approach the metal oxide surface. When the oxide layer becomes thicker, the electric field is reduced since the applied voltage is constant. So the process is self-limiting and a finite compact oxide layer is formed. And Ti^{4+} ions arriving at the interface will

precipitate. In the presence of fluoride ions, water-soluble ion $[\text{TiF}_6]^{2-}$ can be formed according to reaction (2.2). The small ionic radius makes them suitable to enter the growing oxide lattice and transport through oxide by the applied field. The Ti^{4+} ions can be solvated to $[\text{TiF}_6]^{2-}$ (reaction 2.3) to avoid precipitation. The tube formation is started with the surface morphological fluctuations from local field distribution. The initial pore is due to the field-enhanced dissolution. Pores are expanded through a steady-state condition and tubes are formed with small pores vanishing. During the process, oxide is growing on the bottom of the pore and chemical dissolution happens at the top simultaneously [53, 56]. The competition between oxidation and dissolution exists through the whole fabrication process. Nanotubes are formed during the period when oxidation process is faster than dissolution process. Methods can be found to accelerate oxidation or to slow down dissolution, in order to promote the growth rate of nanotube. However, if the anode or the substrate is left in the electrolyte for enough time, due to the composition change of electrolyte, the etching process will eventually become faster than oxidation, which could cause all formed nanotubes to be dissolved.

Another big advantage of electrochemical anodization technique is that the tube structures such as tube morphology, length and diameter can be modified easily by adjusting experimental parameters such as electrolyte, experimental time and applied DC voltage [53, 56, 57, 61].

2.1.2 Fabrication of carbon material by CVD method

Thermal chemical vapor deposition (TCVD) is a widely available technique [164, 165]. It is a chemical process that produces high performance solid

materials. In a typical TCVD process, desired deposition is produced by exposing substrate to precursors (here is the carbon source), which will decompose or react on the substrate surface. The desired deposition is controlled mostly by the reaction temperature, which will affect the reaction of the precursors. Other factors, such as heating rate, gas flow rate, catalyst on substrate also take part on the affection of the growth.

The horizontal tube furnace is one of the most popular setups for TCVD implement. The furnace used for the temperature control is a Thermo Scientific Lindberg M mini-mite tube furnace. The diagram in Figure 2.2 shows the experimental setup. The substrate with catalyst deposited on surface is put in the quartz tube. The carbon source, in this work is ethanol (99%), is brought into the tube by the gas flow via bubbler. Ethanol is decomposed at high temperature and provides carbon source which is needed for the deposition. The gas flow rate can be used to control the amount of carbon that being brought into the tube for reaction. The bigger the gas flow, the more carbon is brought in. Various carbon materials such as CNT, graphene and graphite can be grown by controlling of gas flow, reaction temperate and catalyst used.

Figure 2.2 Schematic diagram of the TCVD used in this work.

In order to improve the deposition quality, different tubes are used for different types of fabrications. Furthermore, before each deposition process, the tube is purged with Ar for 10 to 15 minutes' time to fully evict air and any other gas residues in the quartz tube.

Besides TCVD, another CVD method used in this work is PECVD (Plasma Enhanced CVD). PECVD is used for the fabrication of first order CNT in 3D network structure (Chapter 6). It is a bottom-up growth method. During PECVD process, carbon atoms are introduced by dissociation of carbon-source (C_2H_2 and CH_4). The carbon atoms diffuse into catalyst particles and eventually reach a saturation state. After, carbon will precipitate into tubular forms, grow up in a bottom-up direction and form CNT. The system used is Aixtron Black Magic. A silicon dioxide barrier is used between the silicon substrate and nickel catalyst. C_2H_2 is used as carbon source and NH_3 is the process gas which helps to remove excess catalyst and promote reaction.

2.1.3 Protein detection by ELISA method

In order to read the amount of protein released for the protein delivery test, ELISA is used. It is an easy method to detect the presence of an antibody or a protein in a sample [166, 167]. In ELISA, a microplate is usually used as surfaces for affixed antigen. Then the supplied antibody is applied to bind to the antigen. This antibody is linked to an enzyme. By adding in certain substance, the enzyme can convert to some detectable signal, usually a color change. In this work, the protein is BMP-2 and the ELISA method used is a sandwich ELISA. The BMP-2 used is Recombinant Human BMP-2 (355-BM) and the

ELISA kit used is BMP-2 Immunoassay-DBP 200. Both are from R&D Systems (USA).

Figure 2.3 Procedure diagram of sandwich ELISA process. (1) Affixed capture on plate, (2) sample is added and bind to capture, (3) conjugate is added and bind to sample, (4) substrate is added and converted by enzyme to detectable form.

Figure 2.3 indicates the sandwich ELISA procedures: (1) the capture antibody is bound to the supplied microplate and ready for using; (2) 50 μL sample or standard BMPs with 100 μL assay diluents is added in and bound to the capture; (3) conjugate or the detecting antibody of 200 μL is added in and bound to sample; (4) 200 μL substrate is added in and converted to a color detectable. Then the microplate is incubated for 30 min protected from light, after which 50 μL stop solution is added. The microplate needs to be incubated for two hours at room temperature on a horizontal orbital microplate shaker (Grant-bio PMS-1000) set at 500 rpm $\pm$ 50 rpm after both process (2) and (3). After incubation, the microplate is washed four times to remove any unbounded protein or antibodies.

In order to do a quantity analysis, a standard curve must be generated and used for further calculation of the samples. A series of BMP-2 standards are produced. The concentration used for the curve generation are: 4000 pg/ml,

2000 pg/ml, 1000 pg/ml, 5000 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml. The absorbance of standards is read at 450 nm with microplate reader (Tecan Genios), which is introduced in the characterization section of this chapter. The standard curve is created by computer fitting (Origin). Figure 2.4 shows the generated curve, which will be used in this thesis for calculation of protein concentration. There are two types of standards prepared and read, by PBS buffer which is used for protein delivery and by calibrator dilute which is provided by the kit (Figure 2.4(a)). The average is calculated as the final standard reading. The formula generated and used for further experimental calculation is:

$$\log_{10}X = 1.8149 \times \log_{10}Y + 5.7247, \quad (2.4)$$

where X is the protein concentration and Y is the optical absorbance density.

All the standards and samples are duplicated and read for twice or three times. Average is taken and zero standard optical density is subtracted for all samples to increase the accuracy.

Figure 2.4 BMP-2 standard curves: (a) BMP-2 standard by PBS buffer and calibrator dilute, (b) Standard curve and formula.

2.1.4 Photocatalysis Test

The photocatalytic properties of TiO₂ nanotube and TiO₂-Carbon composite are tested by decomposition of the organic dye, which is Methylene Blue (C₁₆H₁₈ClN₃S). MB is analogy to harmful organic chemicals.

This test is done in a photocatalysis black box which is designed as shown in Figure 2.5. Samples and solutions are placed in sealed Petri dish which is transparent to UV or Visible radiation. The UV light used in this installation is five UV lighting tubes (Philips T5 model, 8W tube with 300 mm in length and 16 mm in diameter) which are arranged in parallel to each other. The UV irradiation ranges from 315 nm to 400 nm with peak wavelength at 370 nm. Same number and size of fluorescent tubes (OSRAM-L823, 8W) are used for visible light testing and the irradiation ranges from 425 nm to 725 nm. The distance between light source and samples is fixed at 35 cm for all photocatalytic experiments. Experiments are taken place in air condition without gas purging or stirring. In this study, different concentrations of MB have been tried. And it is found that degradation of dye can be hardly observed for too high concentration, as sample size is comparably small. The final dye solution is fixed at concentration of 8×10^{-4} g/L, as the photocatalytic efficiencies is more obvious to observe at this concentration. In order to reduce inaccuracy, same amount of MB solutions, 60 ml of 8×10^{-4} g/L concentration, is used for all samples. Sample sizes used are 1 cm × 2 cm for pure TiO₂ samples and 1 cm × 1cm for TiO₂-carbon composite samples. At staggered time intervals, 5 ml of solution is collected at 0, 6, 24, 30, 48, 53 hours for further testing.

Figure 2.5 Schematic of photocatalysis black box

2.2 Characterization Techniques

2.2.1 Electron Microscope

Scanning electron microscope (SEM) is widely used for high resolution images of sample morphology and topology. The SEM makes use of a highly energized electron beam to strike the sample surface. Various signals such as secondary electrons, back scattered electrons and characteristic X-rays are produced from the strike. After bombardment, these signals are captured and converted into image analysis. Secondary electrons are captured and converted into surface morphology and topology images [168]. Electrons have collisions with other electrons and being back-scattered or reflected out of sample contact area. These electrons are back-scattered electrons and will appear bright in the image for high atomic number element. Characteristic X-rays are formed by electrons jumping between element's outer shells and they are captured for EDX analysis which indicates elemental composition of samples [169].

In this thesis, a conventional SEM (JSM-5910LV, Jeol) is used extensively. Electrons are thermionically emitted from tungsten cathode with accelerating

voltage of 15 kV to 20 kV. EDX attached to conventional SEM is used for elemental analysis of samples. Another SEM (JSM-6349F, Jeol) is also used for advanced high resolution. In this SEM, electron is emitted via cold field emission.

Besides SEM, a transmission electron microscope (TEM, TEM 2010, Jeol) is used for atomic scale analysis of samples. TEM uses a high energy electron beam to impinge upon samples to provide more detailed information. The preparation of the specimens for TiO₂ nanotube is done by scratching sample surface, dissolving the nanotubes into acetone under ultrasonication and doping the solution to copper grids.

2.2.2 Raman Spectroscopy

Raman spectroscopy is based on the detection of vibrations on the process of Raman scattering which is due to the inelastic light scattering of photons [170]. Information on chemical structures and physical forms can be provided. Raman is a popular technique, as it can be used to exam materials in solid, liquid or even gaseous, in hot or cold, in bulk, film or even particles. Sample can be simply presented to a microscopy. Incident laser, which can be visible, near infrared or near ultraviolet range, shoots to the sample and a charge coupled device detector is used to collect light after scattering or reflection. Raman data is usually given as wave-number shifts from the incident radiation. The shifts are identifiable for different materials or molecules hence Raman is usually used as a method to identify various material types by their unique pattern of peak positions and relative peak heights.

In this work, the Raman spectroscopy used is confocal Raman Microscopy Alpha300R from Witec. The excitation laser used is a green visible laser with wavelength of 532 nm. The interested range of Raman shift is from 100 to 4000 cm^{-1} . The two most common phases of TiO_2 have peaks ranging from 100 to 1000 cm^{-1} . Anatase has strong Raman wave-numbers denoted at 144, 399, 526, and 640 cm^{-1} [171], rutile has major peaks around 143, 247, 448, 612 and 827 cm^{-1} [172]. Major peaks of carbon materials range from 1000 to 3000 cm^{-1} . The main features in Raman spectra of carbons and CNT lie around 1560 and 1360 cm^{-1} [173, 174]. While the major peaks of graphite and graphene are at 1650 and 2700 cm^{-1} [175, 176]. The peak intensity ratio and peak width can be different for different carbon materials.

2.2.3 Contact Angle Measurement

Contact angle (CA) measurement is a simple technique for surface characterization. It is used to obtain surface energy and to determine surface wettability, whether a solid surface is hydrophobic or hydrophilic. The CA measurement is usually done by applying a liquid droplet on the solid surface and the contact angle is measured from the captured image.

The contact angle (θ) is determined by the balance at the boundary of the three phases (air, liquid and solid). Examples for contact angle of a hydrophilic and a hydrophobic surface are shown in Figure 2.6. When the angle is smaller than 90 degrees, the surface is hydrophilic [177] (Figure 2.6(a)). On the other hand, if the angle is larger than 90 degrees, it suggests a hydrophobic surface [177] (Figure 2.6(b)). The smaller the angle, the more hydrophilic is the

surface. Surface wettability can be affected by surface conditions such as surface roughness and surface heterogeneity.

Figure 2.6 Contact angles of hydrophilic (a) and hydrophobic (b) surfaces.

Dataphysics OCA 20 is the system that was used for contact angle measurement. The sessile drop method is used, in which droplets are introduced to a surface by dispersing liquid through a microsyringe. A video camera is used to capture images of the droplet on solid surfaces. The liquid used is de-ionized water and the volume of the droplet is controlled to be around 4 μL .

2.2.3 Microplate Reader

Microplate readers are mainly used to detect biological events of samples in microtiter plates. It acquires and processes a batch of biological or biochemical data from measuring a colorimetric change. Plate reader is widely used in drug discovery and life science research. There are several detection modes: absorbance, luminescence, fluorescence polarization and fluorescence intensity.

Absorbance mode is used for protein reading in this thesis. In absorbance mode, a source illuminates a single wavelength to the microtiter plate and the light is collected on the other side of the wells. The amount of light that transmitted through the samples are measures and typically related to the concentration of interested proteins or molecules in the samples. A Tecan

Genios microplate reader and a Bio-RAD Benchmark plus microplate spectrophotometer are both used to read proteins. The excitation line used is 450 nm with three seconds' shaking to mix samples before measurement. Before measurement of experimental samples, a set of standards need to be read and used for further calculation of protein amount in samples. The generation of standard curve was presented in the section 'ELISA' earlier in this chapter.

2.2.4 UV-Vis Spectrophotometer

UV-Vis spectrophotometer is used in analytical chemistry for determination of analysis such as organic compounds. The analysis is usually carried in liquid form. The equipment illuminates light of visible and adjacent (near UV and near-infrared) range. The intensity of light passing through a liquid sample (I) is measured then compared to light density that passing through a reference sample (deionized clear water) (I_0). Transmittance ($T = I/I_0$), absorbance ($A = -\log (\%T / 100\%)$) are calculated and they are directly affects the perceived color of the chemicals in solution[178]. Absorbance mode is used in this work and a higher absorbance indicates darker color of the sample hence higher concentration. The system used is Shimadzu UV-2450 UV-Vis spectrophotometer. Absorbance is measured for photocatalytic test and the excitation range used is from 200 nm to 900 nm.

The photocatalytic efficiencies used in this report are relative efficiencies. The relative efficiencies are calculated relative to reference samples and under given set of conditions as described in Section 2.1.4. As mention earlier, MB is used for the photocatalytic test and Figure 2.7(a) illustrates the typical spectrum for MB. Absorbance data used in this thesis is the highest peak area from 520

nm to 720 nm (A , peak center 664 nm) from the absorbance spectroscopy. All data collected from different time intervals are normalized with respect to the initial MB concentration of 8×10^{-4} g/L (A_0 , peak area at 664 nm). The final absorbance used is the relative absorbance A/A_0 , which is linearly related to concentration of MB (C/C_0 , where C is the concentration at the collected time interval and C_0 is the original concentration of MB. detailed discussion is in Chapter 5). Hence, the relative absorbance could be directly used as the relative concentration of MB. The photocatalytic property is better if the relative absorbance or concentration is smaller. An example of photocatalytic data plot is shown in Figure 2.7(b). The data used for photocatalytic efficiency is usually the last collected data point: in this example is the data at 54 hours. Data directly presented in figure is the relative concentration (C/C_0). The photocatalytic efficiency (PE .) used in this thesis is $1 - C/C_0$ in percentage. As shown in Figure 2.7(b), the photocatalytic efficiency is 2.9 % for sample A and 43.4 % for sample B. Sample B presents higher photocatalytic efficiency. The enhancement of the photocatalytic efficiency of sample B as compared to sample A is calculated as: $(PE_B - PE_A) / PE_A = (43.4 - 2.9) / 2.9 = 13.96 = 1396 \%$. Hence the photocatalytic efficiency (also known as decomposition percentage) of sample B is improved by 1396 %. This calculation method will be used in both Chapter 5 and Chapter 6 for the discussion of photocatalytic property of titanium dioxide samples.

Figure 2.7 (a) Example of UV-Vis spectrum for MB, (b) example of photocatalytic efficiency plot.

Chapter 3 Fabrication of TiO₂ Nanotube Structures

3.1 Introduction

Fabrication of highly ordered and self-assembly nanotube or porous TiO₂ structures was found in 1999 by electrochemical anodization [55]. The essential condition for nanotube formation is the existence of fluoride ion in the electrolyte, which is important for Ti metal dissolution and tube shape formation [53, 179]. Without the fluoride ion, the produced layers are disordered and compact. For TiO₂ nanotube fabrication, there are four generations as mentioned in chapter 1, depending on their electrolyte they are named (1) aqueous electrolyte (using mainly HF), (2) buffered electrolyte (pH of electrolyte buffered with neutral chemicals such as NaF and KF), (3) fluoride ion contained organic solvent (using organic chemicals instead of aqueous solution) and (4) non-fluoride electrolyte (using chemicals such as HCl, H₂O₂). In this thesis, we are using organic electrolyte since the nanotubes fabricated from this generation have the most variety in tube morphology and most elasticity in tube diameter and length.

Many researchers have been studying various methods to tailor the morphology of TiO₂ nanotube in order to fit in for various applications including gas sensor, photocatalysts, tissue engineering, solar cells and so on [26, 58, 180-188]. Parameters of TiO₂ nanotube such as tube diameter, tube length, tube density can be adjusted by altering experimental factors such as anodization voltage, experimental time, environmental temperature and solution

composite (in particular the fluoride ion concentration and water content) [63, 68, 189]. Morphology of nanotube can influence its performances in applications. The as fabricated TiO₂ nanostructures are normally amorphous phase. It can be transferred further to crystallized anatase phase by annealing at 450 °C and to rutile phase by annealing at 750 °C or higher [190, 191]. Currently, highly ordered TiO₂ nanotube arrays can be easily fabricated with diameters ranging from 55 to 300 nm and length up to several hundreds of micrometers [53, 189, 192].

In this chapter, various TiO₂ nanotube structures are fabricated using electrochemical anodization technique with organic electrolytes. The effect from different fabrication factors is briefly discussed. Furthermore, this chapter also investigated on the method to fabricate tubes with diameters larger than 300 nm and double-walled TiO₂ nanotubes. Samples are mainly characterized using SEM for morphology and Raman for phase.

3.2 Experimental

Fabrication with various organic electrolytes

TiO₂ nanotube was prepared by an electrochemical process. The anodization was done with a two-electrode cell at room temperature. Pure Ti foil (Sigma-Aldrich; 250 μm thick, 99.7% purity) was used as both anode and working electrode. It was cut into 2 cm × 2 cm size and cleaned by ultrasonication in a mixture of isopropyl alcohol and acetone solution, followed by a thorough rinse with DI water and dried with N₂ gas gun. Pt wire (Alfa Aesar, 1.0 mm diameter, 5 cm length, 99.95% purity) was used as cathode.

Three types of organic electrolytes were used and the volume is fixed at 30 ml. The first electrolyte used was diethylene glycol (DEG, 99%, Sigma Aldrich) with 1% hydrogen fluoride (HF, 48% aqueous solution, Sinopharm Chemical Reagent). The second electrolyte was dimethyl sulfoxide (DMSO, 99.5%, Sinopharm Chemical Reagent) with 2% HF. The third electrolyte was Ethylene glycol (EG, 99%, Sigma Aldrich) with 2% water and 0.3% ammonia fluoride (NH₄F, 40%, Sigma Aldrich). The distance between anode and cathode is 2 cm. The anodization voltages used were 40 V and 60 V. TiO₂ nanotube grew on both sides of the Ti foil after anodization and was washed with isopropyl alcohol and DI water for several times to clean the residual chemicals then dried with N₂ gas. The nanotubes were annealed at 450 °C and 750 °C for phase transformation.

Fabrication of larger diameter nanotube

After the first anodization process, the DEG-HF electrolyte was continuously used with a new anode Ti foil under higher DC voltage (90 V and 100 V). TiO₂ nanotubes fabricated were cleaned and dried following the same routine.

Fabrication of double-walled structure

The double-walled TiO₂ nanotubes are transformed from single-walled nanotubes. The initial single-walled sample was fabricated with titanium foils (purity 99.9 %) of 50 µm thickness. It was anodized in 0.3 % NH₄F solution in EG with 2 vol. % of water at the electrolyte temperature of 0 ± 3 °C. The anodization voltage was linearly increased from zero to a maximum in the

range of 60 – 110 V with the rate of 1 V/s and then kept constant for the total anodization time of no more than 60 min. The anodic current is observed increasing first followed by decreasing due to the reaction in electrolyte. The experiment was ended when the anodic current was below 30 % of its maximum value, for all double-walled nanotube experiments for ease reference. The samples were annealed at 450 °C for 60 min in air atmosphere.

Characterization

SEM at 15 kV accelerating voltage was used to examine the TiO₂ nanotube microstructure. The cross section and bottom view of TiO₂ nanotube arrays were performed by attaching mechanically broken TiO₂ nanotube arrays to carbon conductive taps. Raman is used for material phase examination. EDX is used for checking element composition.

3.3 Result and Discussion

3.3.1 Fabrication of TiO₂ nanotube by anodization with organic electrolyte

As mentioned earlier, tube diameter, length and morphologies can be easily tailored by adjusting experimental parameters. The key factors that control tube structures are electrolyte composition, experimental time and anodization voltage.

Figure 3.1 shows SEM images of top and side view of as-grown TiO₂ nanotubes with DMSO-HF electrolyte, DEG-HF and DEG-NH₄F electrolyte at same anodization voltage of 60 V. Significant difference in tube morphologies can be observed. DMSO-HF electrolyte produces tubes with bamboo structure

on the outer wall (Figure 3.1(a)). On the top section, tubes are helical and bundled together, which could be due to the longer explosion to electrolyte resulted from heavier etching. If the solvent is changed to DEG, outlooks of the tubes are similar but density is smaller with larger tube interspace (Figure 3.1(b)). With DEG- NH_4F electrolyte, the fabricated TiO_2 nanotubes have smaller diameter, smooth outer wall and are closely packed with almost zero interspace (Figure 3.1(c)).

Figure 3.1 SEM images of TiO_2 nanotube fabricated at 60 V for (a) DMSO with 2% HF, (b) DEG with 1% HF, (c) DEG with 0.3 wt% NH_4F . Top view (left) and cross section view (middle and right).

The comparisons on tube diameter, length and growth rate are listed in Table 3.1. For processes (a) and (b), the fluoride ion supplier are both HF but solvent used are different. The tube length from DMSO-HF electrolyte (25 μm)

is much longer than that from DEG-HF (5 μm) and the fabrication rate is several times faster (0.35 $\mu\text{m/h}$ compared to 0.054 $\mu\text{m/h}$). This difference could be introduced by the solvent difference, DMSO ((CH₃)₂SO) is more viscous than DEG ((HOCH₂CH₂)₂O). The existence of –OH in DEG can react with HF and provide extra water content in electrolyte. Hence the PH in DEG-HF electrolyte is more alkaline. The much smaller growth rate could be caused by faster dissolution rate due to the higher water content. The diameter from DEG-HF (300 nm) is slightly bigger than that from DMSO-HF (200 nm). This could also be introduced by solvent difference, DEG has similar molecular structure with DMSO but the chain in DEG (– CH₂CH₂OH) is longer than that (–CH₃) in DMSO. The difference in size could affect the initial formation of tube shape hence affect the diameter. Process (c) is very different from (a) and (b), as the fluoride ion supplier is NH₄F whose pH is much higher than that of the acidic HF. The growth rate for NH₄F-DEG electrolyte is 7.5 $\mu\text{m/h}$ which is over 100 times faster than that for DEG-HF electrolyte. The diameter (100 nm) is only half of that fabricated from DEG-HF electrolyte. Tube configurations can be changed greatly if the electrolyte composition is changed. The difference introduced by the different fluoride supplier is much bigger than that by different solvent.

Table 3.1 Comparison of TiO_2 nanotubes fabricated at 60 V with different electrolyte.

#	Electrolyte	Duration	Diameter	Length	Growth Rate
a	HF +DMSO	72 hr	200-300 nm	25 μm	0.35 $\mu\text{m}/\text{h}$
b	HF +DEG	92 hr	300-350 nm	5 μm	0.054 $\mu\text{m}/\text{h}$
c	NH_4F +DEG	2 hr	100-150 nm	15 μm	7.5 $\mu\text{m}/\text{h}$

The anodization time has control on tube length and wall thickness with other experimental parameters being kept constant [56]. Figure 3.2 shows cross section of TiO_2 nanotubes fabricated in DMSO-HF electrolyte for 24 hours and 72 hours respectively. Tube is 4 μm for 24 hours' fabrication and 10 μm long for 72 hours. Longer duration naturally results in longer tubes. However, the relationship between tube length and duration is not linear. As time goes on during fabrication, configuration of electrolyte such as PH and viscosity changes which affects the oxidation and dissolution process. Oxidation is slower and dissolution is faster. Furthermore, the top portion of tubes have thinner walls compared to the bottom portion, as tube tops are immersed in electrolyte for longer time hence more etching [56, 193].

Figure 3.2 SEM images of TiO_2 nanotubes fabricated for 24 hours (a) and 72 hours (b).

Anodization voltage is the key factor for nanotube growth in electrochemical method if the same electrolyte is used. In DEG-HF electrolyte,

diameter of nanotube is around 200 nm (Figure 3.3(a)) with 40 V DC supply and 300 nm (Figure 3.3(b)) with 60 V. It can be noticed from the SEM images that the larger the potential the bigger the tube diameter. Tube diameter is approximately linearly related to anodization voltage [53]. Some researchers suggested that the anodic oxide starts from a local oxide breakdown site and the growth takes place in all directions from this point with a radius R . As the voltage is applied mainly in the vertical direction, after the initial stage, repeated breakdowns continuously take place at bottom and eventually lead to a tube. The radius is proportional to potential by:

$$R = f_{\text{growth}} \cdot U, \quad (3.1)$$

where R is the radius, U is the potential or anodization voltage and f_{growth} is the growth factor. The growth factor calculated is 2.5 nm/V.

Figure 3.3 SEM images of TiO_2 nanotube fabricated at 40 V (a) and 60 V (b).

For most of the electrochemical experiments reported, electrolyte was usually used for only once. Instead of changing the electrolyte after each experiment, we have tried to reuse the electrolyte for more than once. DMSO-HF electrolyte was used for three times with 40 V supply for 76 hours, 72 hours and 24 hours respectively. New Ti substrate was used each time. As shown in Figure 3.4, the lengths of nanotubes fabricated from the three processes are 7

μm , $15\ \mu\text{m}$ and $2.5\ \mu\text{m}$. The growth rates calculated are $100\ \text{nm/hr}$, $210\ \text{nm/hr}$ and $100\ \text{nm/hr}$ respectively. It is very obvious that when the electrolyte was used for the second time, the growth rate is as twice faster than the first time. The possible reason could be resulted from the change of electrolyte. For the second usage, the oxidation process is faster or the dissolution is slower so that the overall growth rate is increased. However, growth rate dropped for the third usage which is probably due to the too fast dissolution rate caused by the highly conductive electrolyte. Furthermore, no significant tube structures was observed from top view (Figure 3.4(c) insert), instead only porous structure was seen. The cross-section indicated nanotubes with rough surface and almost no inter-tube space. All these suggest that the ability of the electrolyte to produce anodic oxide increases for the second usage but drops from the third usage.

Figure 3.4 Cross section images of TiO₂ nanotube fabricated first (a), second (b) and third (c) usage of DMSO-HF electrolyte. Insert in (c) is the top view.

The as fabricated TiO₂ nanotubes are amorphous and can be transferred to anatase and rutile phase by annealing. Figure 3.5 shows Raman spectra of TiO₂ nanotubes fabricated at different temperatures. After annealing at $600\ ^\circ\text{C}$, TiO₂ shows peaks at 144 , 399 , 526 and $640\ \text{cm}^{-1}$ which are the finger print for anatase phase [172]. Rutile peaks at 448 and $612\ \text{cm}^{-1}$ are significant after annealing at $750\ ^\circ\text{C}$.

Figure 3.5 Raman spectra of TiO₂ nanotube annealed at different temperature. * anatase, # rutile.

3.3.2 Fabrication of large diameter TiO₂ nanotube

In anodization, the diameter of nanotubes is linearly related to the applied potential by equation (3.1): $R = f_{growth} \cdot U$. In order to fabricate tubular structures with larger diameter, higher applied potential is required. However, the applied voltage usually has a range limit and we cannot increase the voltage infinitely to get TiO₂ nanotubes with large diameters. When the applied voltage is below the range, growth cannot be initiated due to the weak electrical field, however, when it is beyond the range, the dissolution rate is too high to maintain tube structures. The tube diameter and applied voltage have linear relationships only when the voltage is within the limit range. In HF contained aqueous solution, a typical range is between 4-25 V and the diameter of the nanotubes ranges from 15 nm to 110 nm correspondingly. For less-aqueous solution, such as HF contained organic electrolyte based, the etching rate is

generally slower and the voltage limit can usually increase to 70 V with diameters reaching 350 nm [56].

Figure 3.6 shows the TiO₂ nanotubes prepared with DMSO-HF electrolyte (organic-based) at 100V, beyond the voltage limit. No nanotubes were sighted from the top view (Figure 3.6(a)). However, by viewing from bottom (Figure 3.6(b)) some structures were found to be similar to TiO₂ nanotubes fabricated at lower voltage (Figure 3.6(b) insert). This suggests that nanotubes were actually formed but etched away as a result of higher dissolution rate caused by high supplied potential. As such, it is possible for nanotubes to be fabricated beyond the voltage limit if the oxide rate could compensate the etching rate. High voltage of 90 V was also tried for DEG-HF electrolyte. Situation was quite alike but some short and thin nanotubes can still be observed. This suggests that DEG-HF electrolyte may have slightly higher threshold compared to DMSO-HF electrolyte. Furthermore, at same voltage, DEG-HF electrolyte can produced nanotubes with larger diameter compared to DMSO-HF as mentioned in earlier section. Hence for the following experiment, DEG-HF electrolyte is used for fabrication of nanotubes with larger diameters.

Figure 3.6 Top view (a) and bottom view (b) of TiO₂ sample fabricated in fresh electrolyte.

Insert in (b) is bottom view of nanotube at 60 V.

As reported in Section 3.3.1, using DMSO-HF electrolyte for the second time can improve the growth rate almost twice as that using fresh electrolyte. This suggests more advance of oxidation process compared to dissolution in the second usage of electrolyte [194]. The experiment was repeated with used DEG-HF electrolyte and an enhancement of the growth rate was also observed. Clearly, a new balance point between the oxidation and dissolution process is established which results in faster growth rate in reused electrolyte.

To investigate further, TiO₂ nanotubes were prepared at 60 V with fresh DEG-HF electrolyte, 90 V and 100 V with used electrolyte. Their average diameters were found to be ~350 nm (Figure 3.7(a)), ~550 nm (Figure 3.7(b)) and ~680 nm (Figure 3.7(c)) respectively. At 100 V, it was observed that the density of the nanotubes reduced and interpore distance increased to 1 μ m. Besides that, portions of the TiO₂ nanotubes fabricated from used electrolyte (Figure 3.7(e) & (f)) were etched away and the underlying substrate was exposed. This phenomenon was not observed for TiO₂ nanotubes fabricated at 60 V with fresh electrolyte (Figure 3.7(d)). In general, used electrolyte at higher voltage produces nanotube with larger diameter; it also causes a reduction in density, due to a faster etching rate.

Figure 3.7 SEM images of TiO_2 nanotube fabricated at 60 V in fresh electrolyte (a,d), 90 V (b, e) and 100 V (c, f) in second used electrolyte. Nanotubes in red circle areas were etched away and substrate is exposed.

Process currents of DEG-HF electrolyte used for the first and second time were recorded down for the first 24 hours and displayed in Figure 3.8. While being used for the first time, the starting current was as small as 0.5 mA. This indicates low conductivity of the freshly mixed electrolyte, which is reasonable because majority of the solution is organic solvent and there are less conductive ions. As the anodization going on, more ions were introduced and the conductivity was increased. Current was slowly raised to 2.2 mA for the first day and fluctuated from 2 mA to 8 mA for the following time. When the electrolyte was put for the second usage, there were more ions in solutions hence higher conductivity. The current immediately jump to 40 mA once the cell was connected. After reach 40 mA, the current fell quickly to 2 mA and fluctuated around this number between 2 mA to 4 mA for the following process.

Figure 3.8 Process current with the first and second usage of DEG-HF electrolyte.

Figure 3.9 shows the linear fitting for the tube diameter versus applied potential with equation (3.1) and the growth factor f_{growth} of TiO₂ nanotube was found to be 3.0 nm/V. This value is 20% higher than the average growth rate of 2.5nm/V reported earlier for Ti, as referred at the insert figure [53].

Figure 3.9 TiO₂ nanotube diameter as function of applied voltage in DEG-HF electrolyte.

3.3.3 Fabrication of double-walled TiO₂ nanotube

There are many ways to improve the catalytic properties of TiO₂ materials and increasing the surface area is one of the easiest methods. Double-walled structures could sufficiently enlarge surface area, but they are seldom reported. The main reason is the need for high anodization voltage which normally goes

beyond the voltage limit and makes the process unstable. Here we present an easier fabrication way for the first time by Ti anodization within the conventional regimes of applied.

Besides voltage, electrolyte and experimental time, electrolyte temperature is another important factor to determine peculiarities of TiO₂ nanotube from anodization[195]. Under lower temperature, the dissolution or etching process is suppressed. Lower temperature allows higher limit of anodic voltage. If fabricated under same voltage, nanotubes produced from lower temperature have smaller pore size and bigger wall thickness. Figure 3.10 presents the cross section of TiO₂ nanotubes fabricated at normal room temperature (Figure 3.10(a)) and 0 ± 3 °C (Figure 3.10(b)). The inner pore diameter is half (75 nm / 150 nm) of the outer diameter from fabrication at room temperature and the ratio reduced to a quarter (50 nm / 200 nm) as the electrolyte temperature dropped to 0 °C. The roughly calculated porosity decreases from over 20 % to lower than 5 %.

Figure 3.10 Cross-section SEM images of TiO₂ nanotube fabricated at 25 °C (a) and 0 – 3 °C (b) at anodization voltage of 60 V.

A batch of anodic titania nanotubes were fabricated. The as fabricated tubular structures are typically single-walled even after annealing. The

appearance of double-walled structures only happens to nanotubes with outer diameters larger than 150 nm and porosity smaller than 10 %. Figure 3.11 shows SEM images of the tubular film fabricated by anodization at low temperature followed by subsequent annealing. It can be noticed that the as-prepared tubes are single-walled with diameters of over 200 nm. The nanotubes have very thick walls and very small pore sizes in the middle. In this case, the porosity is usually less than a few percentages. Double-walled structures are evident upon annealing at 450 °C.

Figure 3.11 SEM images of the TiO₂ nanotube structures before annealing (a) and after annealing (b, c). Anodization voltage is 110 V (a) and 75 V (b, c).

Figure 3.12 illuminates the formation of supposed double-walled structures. The as fabricated TiO₂ nanotubes are amorphous (evident from section 3.3.1). Furthermore, from EDX analysis, impurities such as carbon (~10 at. %) and fluoride (~ 6 at. %) are found to be incorporated into the tubes. Impurities in tubes are normally found highest at the tube surface and lowest along the border [196]. The concentration of impurities is shown by a gradient fill in Figure 3.12(a) where darker area means higher contents. Upon annealing, large portion of impurities were released and the percentage decreased obviously: carbon dropped to 3.29 % and fluoride dropped at similar ratio. At the same time, amorphous TiO₂ was transferred to crystalline anatase at 450 °C. As a result,

volume of each tube was decreased and mechanical strains rose in radial direction (Figure 3.12(a)). Since impurities content is higher along the inner tube surface, the decrease of volume happens more on these areas. The outer borders of tubes are tightly bounded and the inner tube surfaces are also mechanically stable because of the round shape. Therefore, the mechanical strains on radial direction could only be released from the middle and split the thick wall into two. Thus, the concentrically arranged inner tube and outer tube are formed which presents us the double-walled nanotube structures.

Figure 3.12 Schematic of formation of double-walled tubular TiO₂: (a) as fabricated nanotube with single-walled structure; (b) double-walled structure upon annealing at 450 °C.

3.3 Summary

In summary, various TiO₂ nanotube structures have been fabricated successfully by adopting anodization method. Various experimental parameters, such as electrolyte, anodic voltage, experimental time and repeated use of electrolyte, were investigated for their influence on tailoring of the tubular structures. Additionally, TiO₂ nanotubes with diameters larger than 350 nm were fabricated successfully by re-using of DEG-HF electrolyte at high supplied potential. Tube diameter of 550 nm and 680 nm were achieved at

anodization voltage of 90 V and 100 V respectively. Finally, double-walled titania tubular structures were successfully fabricated in NH₄F-EG electrolyte. Ti foil was anodized at low temperature to provide tube with thick walls and low porosity. The subsequent annealing split the tubes into two concentric tube and formed double-walled structure. The mainly reason for splitting could be the mechanical radial strains, introduced by release of impurity atoms and volume shrinking during annealing process.

Chapter 4 BMP-2 Delivery with TiO₂ Nanotube Structures

4.1 Introduction

Because of its ability in inducing bone formation, Bone morphogenetic protein-2 (BMP-2) has been widely used in restoration and treatment of orthopedic [84, 197-199]. After administration, large amount of BMPs are either lost or losing their bioactivity because of the fluid, enzymatic activity and so on [87]. Denatured bone growth may be introduced by an improper delivery of protein. Hence, it is essential to have a localized and release-controlled carrier to enhance BMPs' effectiveness and to fulfill different demands in various surgeries [200, 201]. As introduced in Chapter 1, there are a number of carriers such as polymer [202-204] and inorganic materials [205, 206] that have been studied nowadays. The major problem of current delivery system is the too high initial burst which requires more loading amount hence higher cost [207]. Another problem is the release rate, which is usually too fast for efficient bone repair [208]. Until now, there are still various problems and no single clinically-effective delivery system has been developed for BMP-2.

Titanium and its alloys have been long used as implant materials in bone implant surgeries [35]. Instead of Ti, it is usually the thin oxide layer on the surface provides interaction with cell environment. The good biocompatibility and mechanical properties of Ti/TiO₂ materials make them excellent implant materials [34]. Furthermore, the surface of Ti/TiO₂ can be easily modified with nanostructured coatings by electrochemical anodization as introduced in

Chapter 3. The nanostructured surfaces usually have different roughness and surface energy which have further influences on protein loading and release [209]. Nanomaterials have got much attention in bio-area, because of their superiority in terms of longer residencies in circulation and extending of the bioactivity of the entrapped molecules [114, 115]. We also hypothesize that the tubular structures can provide more storage space for proteins and might improve protein delivery with slower release rate.

In this chapter, we have investigated BMP-2 delivery with various TiO₂ nanotubes and studied their influences on protein elution from different aspects of nanotubes such as morphologies, diameters, length and phases.

4.2 Experimental

Fabrication of TiO₂ nanotubes with different morphologies

TiO₂ nanotubes were prepared by electrochemical anodization method described in Chapter 2. Nanotubes with different morphologies were fabricated at 60 V in DEG-HF, DMSO-HF, DEG-NH₄F electrolyte. Experimental durations were 72 hours, 24 hours and 40 minutes respectively. The Ti reference sample was prepared by placing the fabricated nanotube sample into a high power ultrasonic bath to fully remove the TiO₂ nanotube on surface. The rough sample with rougher surface was prepared through an anodization process at 20 V for 24 hours.

Fabrication of TiO₂ nanotube with large diameter

DEG-HF electrolyte was used to produce TiO₂ nanotubes with different diameter. Normal TiO₂ nanotube sample was fabricated in fresh electrolyte at 60 V for 72 hours. The sample with larger diameter was prepared with fresh Ti foil in the above used electrolyte from the previous process at 90 V for 72 hours.

Fabrication of TiO₂ nanotubes with different lengths

To study the influence on protein delivery from different tube lengths, samples were prepared in the same DEG-HF electrolyte at 60 V potential for different experimental duration. Experimental time of 24 hours (fresh electrolyte), 24 hours (used electrolyte) and 72 hours (fresh electrolyte) were used to fabricate nanotubes with different lengths. A Ti reference and Ti rough samples were used as references for non-tubular structures.

Fabrication of TiO₂ nanotubes with different phases

All the above mentioned nanotubes are amorphous without any annealing. In order to study the influence on protein delivery from different material phases, a Ti reference sample and nanotube sample (DEG-HF at 60 V for 72 hours) were fabricated and annealed. The as prepared amorphous samples were annealed using TCVD system in Ar atmosphere for one hour at 600 °C and 750 °C to produce anatase and rutile phase respectively. The ramping up rate used is 30 °C / min.

Characterization

SEM with 15 kV accelerating voltage and 15 mm working distance was used to study the TiO₂ tubular structures. Mechanically broken nanotube arrays were attached to carbon conductive tapes and observed for cross section and bottom view. Raman is used to examine material phase. Surface wettability of TiO₂ samples was examined using water on contact angle equipment with sessile drop method.

BMP-2 delivery

All the samples were cut into the same 1 cm × 1 cm size, sterilized with 70 % ethanol followed by exposure under UV light in a bio safety cabinet (BSC) for several hours. The sterilized nanotube samples were then put into a twenty four well culture plate and loaded with 20 µL of BMP-2 with concentration of 10 µg/l (solvent used is 4 mM hydrogen chloride with BSA no less than 0.1 %). The total loaded protein amount is 200 ng. The plate was protected from light and dried naturally overnight. After drying, 1 ml of 1× phosphate buffer solution (PBS) with 0.1 % BSA were put into wells with samples and incubated at 37 °C. The buffer solutions were replaced at time intervals of 10 minutes, 1, 2, 4, 8 hours and 1, 3, 7 days. The collected solutions were kept in 1.5 ml vials and stored at -20 °C until next usage.

ELISA was used as the method to examine protein concentration in the collected solutions. Microplate of ninety six well was used and the absorbance was read with microplate reader at 450 nm. Three readings were made for one solution sample and an average was taken to reduce unnecessary errors. The

exact protein concentrations were calculated from the standard curve and equation (2.4): $\log_{10}X = 1.8149 \times \log_{10}Y + 5.7247$, where X is the protein concentration and Y is the absorbance obtained from microplate reader. All data shown in this chapter were normalized for comparison.

4.3 Result and Discussion

Titania tubular structures were fabricated and tested for their influences on BMP-2 delivery. This section discussed the delivery results from nanotubes with different morphologies, different diameters, different lengths and different material phases respectively. BMP-2 deliveries were all processed under the same procedure.

4.3.1 BMP-2 delivery with TiO₂ nanotubes of different morphologies

In order to compare protein delivery function, two samples with flat surfaces: Ti substrate and rough surface; three nanotube samples: DEG-NH₄F NT, DMSO-HF NT and DEG-HF NT, were fabricated with respective electrolytes. The morphologies of nanotubes vary with the types of applied electrolytes.

Figure 4.1 shows the SEM images of all samples. It could be noticed that Ti substrate (Figure 4.1(a)) is smooth and with no tubes left on surface after the ultrasonication process. The rough surface sample has an anodized surface at low voltage with no tubes as well (Figure 4.1(b)). On the other hand, all the nanotube samples have surfaces filled with tubes. DEG-NH₄F NT is more like a porous film with tubes highly packed together, which is more similar to alumina porous structure (Figure 4.1 (c,d)). The diameter is comparably small,

around 100 – 150 nm. DMSO-HF NT is still bundled together in smaller areas and have relatively big space between bundles (Figure 4.1 (e,f)). The nanotubes have diameters at 200 – 250 nm. Figure 4.1 (e,f) displays the top view and side view of DEG-HF NT. It can be seen that the nanotubes are highly ordered and well separated with uniform spacing. The average diameter is 300 – 350 nm which is the biggest among all three nanotube samples.

Figure 4.1 SEM images of fabricated samples. Top view of Ti substrate (a) and rough surface (b). Top and side view of DEG-NH₄F NT (c, d), DMSO-HF NT (e, f) and DEG-HF NT (g,h).

The difference in morphology certainly affects surface properties. The surface wettabilities of samples were evaluated using contact angle. If the contact angle is higher than 90 degrees, a hydrophobic surface is achieved. Lower than 90 degrees means hydrophilic surface. Generally speaking, hydrophilic surface suggests better biocompatibility and more preferred in bio-related applications. Figure 4.2 indicates the contact angles of all five samples. Both Ti substrate and rough surface samples demonstrate contact angle of 70 degrees (Figure 4.2(a,b)). The DEG-NH₄F NT has smaller angle of 20 – 30 degree (Figure 4.2(c)). DMSO-HF NT and DEG-HF NT both have contact angle of a few degree (Figure 4.2(d,e)). DEG-HF NT has almost zero degree.

Figure 4.2 Contact angle of Ti substrate (a), rough surface (b), DEG-NH₄F NT (c), DMSO-HF NT (d) and DEG-HF NT (e).

It is quite obvious that all samples have contact angles smaller than 90 degrees hence all surfaces are hydrophilic. Furthermore, all nanotube samples have angles smaller than samples with no nanotubes, indicating higher hydrophilic property of nanotube structures. Lastly, the zero contact angle of DEG-HF NT reveals the superhydrophilic property of the sample. The order of contact angle can be put in descending order as: Ti substrate > rough sample >

DEG-NH₄F NT > DMSO-HF NT > DEG-HF NT. The order of surface hydrophilicity follows exactly the opposite direction. Surface microstructure, material composition and chemical property can all contribute to affect surface wettability [210]. Here, both Ti substrate and rough surface samples allow water to sit on them. With surface modified by nanotubes, water droplet could disperse into the gaps easily by capillary action [211] which induces higher hydrophilic property.

The surface wettability showed its influence on protein delivery as early as on the very beginning of loading process. Protein solutions quickly dispersed and penetrated into DEG-HF NT and DMSO NT surfaces but demonstrated a small contact angle on DEG-NH₄F NT sample. Solutions sat on for both Ti substrate and rough surface samples; in order to spread the solution to whole surface, a tip was used to direct solution to edges. These phenomena are well fitted with the surface wettability: a more hydrophilic surface absorbs protein solution faster. Naturally, the drying process was faster for nanotube samples.

Figure 4.3(a) demonstrates the cumulative release profiles of BMP-2 for all samples. Ti substrate and rough surface samples eluted almost 100 % protein with more than 95 % released in the first few hours. On the other hand, nanotube structures eluted only a few percentages and held more than 95 % protein. The expected main reason would be the tubular structure, which provides not only more binding sites but also stronger interaction between nanotube and protein. Proteins could bind tightly with nanotubes through van der Waals and electrostatic forces [212]. The increase on binding sites is introduced by the enlarged surface area with the tubular structure. Furthermore,

due to the superhydrophilic property of nanotubes, protein solution could wet the entire surface and sit between or into nanotubes.

The protein elutions from Ti substrate and rough surface for the first few hours were compared in Figure 4.3(b). It can be observed that the rough surface sample has slower elution rate. This could be introduced by the difference in surface roughness. The delivery profiles for nanotube structures were shown in Figure 4.3(c). The total released protein amount could be put in order of; DEG-NH₄F NT > DMSO-HF NT > DEG-HF NT, the release rate is in the same order. It can be noticed that these results are tightly related to the surface wettability and degree of nanotubes' orders. A more hydrophilic surface elutes less total amount of protein at a slower release rate. This suggested a tighter binding between nanotube and protein for more hydrophilic surface.

Figure 4.3 Cumulative release profile of BMP-2 for (a) all samples, (b) Ti substrate and rough surface sample, (c) TiO₂ nanotubes samples.

The amount of protein released at different time intervals are displayed in Figure 4.4. Both Ti substrate and rough surface samples have initial burst over 80% in the first 10 minutes (Figure 4.4(b)). At the following time intervals, the amount has a decline trend. The rough surface sample has a smaller initial burst (80 %) compared to that of Ti substrate (90%), but larger eluted amount in all the following time intervals. This indicates that the release path of protein is affected by the roughness of the surface. Figure 4.4 (b) exhibits the data for the nanotube samples. Instead of an obvious decline trend, the amounts eluted from nanotube samples are relatively constant with small fluctuations over the entire process. The big obstacle for drug delivery, the large initial burst [96], is now

suppressed significantly to less than 1 %, which further proves the strong interaction between protein and nanotube structures. In a descending order, the amount released at different time intervals for nanotube samples are: DEG-NH₄F NT > DMSO-HF NT > DEG-HF NT. This is consistent with the surface wettability and suggests a tighter binding with more hydrophilic surface.

Figure 4.4 Amount of BMP-2 released at different time intervals of (a) TiO₂ nanotube samples and (b) Ti substrate and rough surface sample.

4.3.2 BMP-2 delivery by large diameter TiO₂ nanotubes

To investigate further, TiO₂ nanotube with large diameters (DEG-HF 100 V NT) were fabricated with used electrolyte at high potential of 100 V after the first usage of the DEG-HF electrolyte at 60 V (DEG-HF 60 V NT). The two set of samples have similar tube length of 5 μm and have been tested for BMP-2 delivery.

The diameter of nanotubes fabricated at 60 V is ~ 350 nm (Figure 4.5(a)) and increased to ~ 680 nm at 100 V (Figure 4.5(b)) in used electrolyte. Despite the increase in tube diameter, tube density for DEG-HF 100 V NT samples is noticed to be decreased. As shown in red circles (Figures 4.5 (d)), portions of the TiO₂ nanotubes were etched away which could be due to the high etching rate introduced by the high potential. However, the exposure of substrate in small areas is not noticed for DEG-HF 60 V NT samples (Figure 4.5(c)). In general, DEG-HF 100 V NT sample has nanotubes with larger diameter but reduced density compared to 60 V NT sample.

Figure 4.5 SEM images and contact angle of DEG-HF 60 V NT (a,b,c) and 100 V NT(d,e,f)

Both NT samples have small contact angle of a few degree (Figure 4.5 (e,f)). This indicates that despite the change on tube diameter and density, the sample surface maintains its superhydrophilic property.

Figure 4.6 shows both the cumulative and separate release profiles. Due to the superhydrophilic property, most of the proteins are held and the total eluted amount is below 5 %. From the amount of protein released at different time intervals (Figure 4.6(b)), it can be noticed that both NT samples have relatively constant amount of proteins released over time. The DEG-HF 100 V NT eluted more proteins at different time intervals over the entire process than 60 V NT. Furthermore, the 100 V NT sample has an initial burst of 2% while 60 V NT sample gives no initial burst.

Figure 4.6 BMP-2 delivery profiles of DEG-HF 60 V and 100 V NT samples. (a) cumulative release profile and (b) amount of protein released at different time intervals.

The initial burst for DEG-HF 100 V NT is still small as compared to that of Ti substrate from last section. The burst could be introduced by the sparse areas where nanotubes were etched away. These areas are not able to hold protein as tight as nanotubes. However, the total areas of the sparse sections are relatively small compared to the whole sample hence the initial burst is not significant.

Another observation is that nanotubes with larger diameters eluted more protein compared to that with smaller diameters at each time interval. This difference may be due to the larger diameter and the lower packing density. The wider diameters allow ease of entering as well as eluting of solvent from the tubes. In addition, some protein may be loaded into the large gaps between tubes which are loosely attached thereby easier to be released. Furthermore, it is noticed in the protein delivery process, TiO₂ nanotubes with larger diameter (fabricated with used electrolyte) shows weaker mechanical strength: a very small portion of nanotubes peeled off from substrate. This suggests that nanotubes fabricated from used electrolyte has weaker mechanical strength, which could be induced from the over etching during anodization process.

4.3.3 BMP-2 delivery with TiO₂ nanotubes of different lengths

To study how the tube length may affect BMP-2 delivery, TiO₂ nanotube samples with different lengths were fabricated from DEG-HF electrolytes. To maintain the same tube diameter, potential of 60 V and DEG-HF electrolyte were used for all fabrication processes.

Ti substrate is used as reference and considered as sample with tube length of 0 μm . Nanotubes with length less than 1 μm was fabricated in fresh DEG-HF electrolyte for 24 hours (Figure 4.7(a)). Nanotube structures just started to form and the growth rate was slow at this stage. The electrolyte was used the second time for 24 hours and nanotube sample with length of 2 – 3 μm was obtained (Figure 4.7(b)). The growth rate was twice (2 $\mu\text{m}/\text{day}$) higher than that for fresh electrolyte (1 $\mu\text{m}/\text{day}$). The 5 μm nanotube sample was prepared in fresh

electrolyte from 72 hours anodization (Figure 4.7(c)). With the same 60 V potential, tube diameters are similar for all nanotube samples.

The contact angles of nanotube (NT) samples with different tube lengths are also shown in Figure 4.7. NT < 1 μm has contact angle of 60 degree, NT ~ 2 – 3 μm has angle of approximately 20 degrees and NT ~ 5 μm has contact angle of 4 degrees. From section 4.3.1, it is known that Ti substrate (corresponding to NT ~ 0 μm) has contact angle of 70 degrees. It can be noticed that contact angle is decreasing with the increasing tube length. This indicates that the surface becomes more hydrophilic with longer nanotubes and meets the superhydrophilic status when the tube length reaches 5 μm .

Figure 4.7 SEM images and contact angles of TiO₂ nanotube samples with different lengths. (a) NT < 1 μm , (b) NT ~ 2-3 μm , and (c) NT ~ 5 μm .

Both the cumulative release profile and amount of protein released at different time intervals are shown in Figure 4.8. The initial burst for Ti substrate is over 80 % and this number is immediately reduced by half to 40 % when the surface was modified with very short nanotubes of length less than 1 μm . With slightly longer tubes of 2 – 3 μm , this number is reduced again to

only 10 %. There is almost no sign of initial burst for 5 μm nanotube sample. The total released protein amount is also reduced accordingly, from nearly 100 % with Ti substrate to 55 % with 1 μm nanotube samples, to 25 % with 2 – 3 μm nanotube samples, and eventually to 5 % with long nanotubes of 5 μm length. It is found that the initial burst and total protein elution is most profoundly affected by nanotube length. With longer tubes, there is more suppression on the initial burst and more protein is trapped by nanotubes. This is within expectation since longer tubes translate to more loading sites for protein. In addition, the increasing hydrophilicity with longer tubes may enhance the penetration of protein which results in tighter binding. All these can be further verified by the amount of protein release at different time intervals. From Figure 4.8 (b), for nanotube samples only, it can be noticed that the amount of protein released is decreasing when length of used nanotubes is longer. All these suggest tighter binding and deeper penetration of protein into longer tubes.

Figure 4.8 BMP-2 delivery profiles for TiO₂ nanotubes with different lengths. (a)

Cumulative release profile, (b) amount of protein released at different time intervals.

4.3.4 BMP-2 delivery with TiO₂ nanotubes of different phases

In addition to diameter and length, elution curves of TiO₂ samples with different material phases have also been studied. Ti substrate and DEG-HF 60 V NT samples were used for the tests. Their duplicates were annealed at 600 °C and 750 °C in Ar atmosphere respectively to transfer the as prepared amorphous to anatase and rutile phases.

Figure 4.9 shows the SEM images of Ti substrate and TiO₂ NT samples before and after annealing. After annealing at 600 °C, the main surface structures retained the same for both samples, except for the TiO₂ NT sample in which the nanotubes tend to bundle. However, there are some structure changes for both samples after annealing at 750 °C. Surface of the substrate sample was fractured with grains (Figure 4.9(c)). While for the TiO₂ NT sample, nanotubes were tending to merge together (Figure 4.9(f)). The open pores at tube top, which can be observed clearly for amorphous nanotube, are not so significant and some tips are even merged together with no sign of pores anymore. Nanotubes are still vertically aligned but with small bundles formed by a few or tens of nanotubes. Despite the annealing process, surface wettabilities are maintained. Substrate samples were hydrophilic with contact angle of 70 degrees and nanotube samples are super hydrophilic with contact angle of almost zero as mentioned in section 4.3.1 earlier. The change of material phase doesn't have big influences on surface wettabilities.

Figure 4.9 SEM images of: Ti substrate with no anneal (a), annealed at 600 °C (b) and 750 °C (c); TiO₂ nanotube with no anneal (d), annealed at 600 °C (e) and 750 °C (f).

Amorphous titania has no obvious peaks and can transfer to crystallized phase by simple annealing. There are two common phases: anatase and rutile which can be distinguished by Raman. Anatase has Raman peaks at 145 cm⁻¹, 198 cm⁻¹, 399 cm⁻¹, 516 cm⁻¹ and 640 cm⁻¹ [172]. While rutile has peaks at 143 cm⁻¹, 448 cm⁻¹ and 612 cm⁻¹ [172]. Raman spectra of the samples are shown in Figure 4.10. Both substrate and TiO₂ NT samples are transferred to anatase phase after annealing at 600 °C and showing fingerprint peaks at 145 cm⁻¹, 399 cm⁻¹, 516 cm⁻¹ and 640 cm⁻¹. Rutile phase is also observed after annealing at 750 °C with peaks at 448 cm⁻¹ and 612 cm⁻¹.

Figure 4.10 Raman spectra of TiO₂ NT (a) and Ti substrate (b) annealed at different temperatures.

During protein loading, protein solvent were quickly dispersed and penetrated into all three TiO₂ NT samples but sat on substrate samples. These phenomena are well fitted with surface wettabilities and consistent with the contact angle tests.

The elution curves are displayed in Figure 4.11. Both substrate and TiO₂ NT samples exhibits similar trends for cumulative release profile (Figure 4.11(a,b)): the total eluted amount of protein and release rate decreased for anatase samples compared to amorphous sample and both numbers were reduced further for rutile phase. For substrate sample, the total released protein reduced by a few percentages in the sequence of amorphous > anatase > rutile,

which is not big if compared to the totally released amount of over 88 %. However, for TiO₂ NT sample, a difference of a few percentages could be big since the total eluted amount is generally smaller than 10 %. From Figure 4.11 (c) and (d), a similar trend was noticed again. At each time interval, protein released from rutile phase is smaller than that from anatase phase and the number from amorphous is the largest of them all.

Figure 4.11 Elution curves of BMP-2 for samples with different phases: Cumulative release profile for TiO₂ NT (a) and Ti substrate (b); amount of protein released at different time intervals for TiO₂ NT (c) and Ti substrate (d).

Even though the annealing process has no significant influence on surface wettability, surface structures exhibits certain amount of change. For substrate sample, cracks and grains are introduced after high temperature annealing. As a result, the surface becomes rough and area is enlarged. A larger surface area provides more loading volume hence can hold more protein and release less.

While for nanotubes sample, bundling and merging of the nanotubes could cause difference on protein loading and release too. From the result, it can be noticed that the merged and bundled nanotubes retain protein better.

4.4 Summary

In this chapter, TiO₂ nanotubes with different outlooks, diameters lengths and phases were fabricated and their influences on protein delivery were studied. In general, Titania nanotubes provide super hydrophilic surface and more loading volume for proteins. The initial burst is significantly suppressed and the elution rate is reduced. Tube diameter and density has obvious effect on protein elution: wider diameter and large gaps allow solvent to enter tubes easier also to elute easier. The elution is most profoundly affected by tube length. Samples with longer tubes are more hydrophilic, provide more loading sites and allow deeper penetration. Hence, there is more protein held by longer tubes and less proteins eluted. The change of phase doesn't affect surface wettabilities but does introduce some structure changes, which has minor influences on the overall elution.

In summary, this study suggests that the TiO₂ nanotube structures can be used as a suitable carrier for BMP-2 release for enhanced bone repair. The BMP-2 elution process could be controlled by TiO₂ nanotube structures with different morphologies. Further studies will be needed to test on BMP-2's delivery efficiency and bioactivity using nanotube structures. Mechanical strength of TiO₂ nanotube coated grafting device could also be an interesting study area.

Chapter 5 Photocatalytic Properties of TiO₂ Nanotube Structures

5.1 Introduction

Titanium dioxide, in its anatase form, is a photocatalyst which is able to carry on photochemical activity. The photocatalytic property could be used for water photolysis or surface oxidation of toxic organic compound, particularly under UV light [41, 213, 214]. It is widely used in air and water purification [215-217], dye-sensitized solar cells [218-220], self-cleaning [221-223] or other applications because of its sterilizing and photocatalytic properties. It can be fabricated using various synthesis methods, and its physical forms can vary from particles, membranes to wires. Many research scientists are focusing on the increase of its photo-efficiency. One major path is to increase titania's absorption range in optical spectrum [224-228]. Doping is usually used to alter its band gap hence shift the absorption wavelength from UV range to visible range [224, 225, 228]. The other way to improve the catalytic property is to increase the surface area which is one of the easiest methods to enhance its decomposition efficiency [229, 230].

Nano-tubular TiO₂ film provides much larger surface area for photo-electrochemical action and has demonstrated higher efficiency than the commercial P25 layer under comparable conditions [152]. Particularly, both the inside and outside walls could be easily accessed by redox couples in electrolyte which provide higher absorption of light. The most popular method to prepare such tubular structures is electrochemical anodization as introduced

in Chapter 3. The morphology and thickness of TiO₂ nano-tubular films could be easily tailored by controlling the experimental factors, such as electrolyte composition, anodization duration, and applied potential. Considering all these properties, Titanium dioxide nano-tubular structures are very worthy to be studied for photocatalytic applications.

In this chapter, we shall present studies on the photocatalytic properties of TiO₂ nanotube structures fabricated from various electrolytes. Both single-walled and double-walled nano-tubular structures were investigated for their application in water purification.

5.2 Experimental

Fabrication of single-walled TiO₂ nanotubes with different morphologies

Three different kinds of single-walled TiO₂ nanotubes were fabricated with anodization method. Potential of 60 V was applied in DEG-HF, DMSO-HF, EG-NH₄F electrolyte for 72 hours, 24 hours and 40 minutes respectively. The as prepared samples were annealed in Ar atmosphere at 450 °C for one hour for the phase transfer.

Fabrication of double-walled TiO₂ nanotubes

The double-walled TiO₂ nanotubes were fabricated in EG-NH₄F electrolyte at low temperature (0 ± 3 °C) followed by annealing for one hour at 450 °C and 600 °C respectively. The total anodization time was less than 60 min and the maximum voltage ranged from 60 V to 110 V with an increasing rate of 1 V/s. The experiment was ended when the anodic current was below 30 % of its

maximum value. The samples were annealed at 450 °C and 600 °C for 60 minutes in air atmosphere. A single-walled sample was prepared as reference. Same electrolyte was used with voltage ranging from 40 – 60 V at room temperature. The annealing was processed at 450 °C for one hour in air.

Characterization

The structure of TiO₂ nanotube was studied by SEM and TEM. TEM samples were prepared by dissolving nanotubes in acetone and putting droplet on copper grids. Raman was used to examine material phases. EDX was used to study the elemental composition.

Photocatalysis test

The photocatalytic properties of TiO₂ nanotubes were studied using the black box that introduced in Chapter 2. Both UV and visible lights were used as mentioned. Sample size used was 1 cm × 2 cm for TiO₂ nanotube samples and 1 cm × 1 cm for double-walled TiO₂ samples. Organic dye solution applied was MB with concentration of 8×10^{-4} g/L. There were no stirring or purge gas involved in the process. Sample solutions of 5 ml were taken out at time intervals of 0, 6, 24, 30, 48, 54 hours. The final absorbance data was obtained from sample solutions with Shimadzu UV-2450 spectrophotometer.

5.3 Result and Discussion

TiO₂ tubular films were fabricated and studied for their photocatalytic properties in water purification. This section presents the decomposition efficiency on MB with various tubular structures.

5.3.1 Methylene blue

Aqueous solution of methylene blue (MB) in this work is used as the organic dye for the photocatalytic tests. MB is a common model pollutant used to evaluate the photocatalytic activity of photocatalyst, which appears to be blue color. The concentration could be easily monitored using a spectrometer. A darker color appears for solution with higher concentration, hence higher absorbance from UV/Vis spectroscopy. During a photocatalytic test, concentration of MB reduces due to the decomposition induced by photocatalyst. As a result, the absorbance of MB decreases as time going on in a photocatalytic testing. How fast the concentration drops suggests how efficient the photocatalyst is.

Figure 5.1(a) demonstrates a model of UV/Vis absorbance spectra for MB in a photocatalytic test. Absorbance of MB was measured from 200 nm to 900 nm and the highest peak is at 664 nm. Absorbance is decreasing as time increases in test with photocatalyst. The absorbance reflects concentration of MB which further indicates the decomposition efficiency of the photocatalyst. Peak area of 664 nm is calculated from 520 nm to 720 nm and used as the absorbance data in this work.

Figure 5.1 (a) UV/Vis absorbance spectra of MB solution collected at different time intervals and (b) calculated peak area at 664 nm at different MB concentrations.

Figure 5.1(b) presents the relationship between the absorbance and MB concentration. Concentration of one means the initial MB concentration of 8×10^{-4} g/L which was used in the photocatalytic test. It can be noticed that the absorbance calculated is linearly related to concentration by $A = k \cdot C$, where A is the absorbance, k is the slope and C is the concentration. This advises that the change in absorbance (A/A_0 , A_0 is the initial absorbance of MB) directly reflects the change in concentration (C/C_0 , C_0 is the initial concentration of MB), and it can be used straightly to indicate the decomposition efficiency. Hence the conversion from absorbance to concentration is not necessary.

To eliminate any error from environmental and MB itself, a batch of experiments were done without any photocatalyst. These data would be employed as the reference for the comparison of photocatalytic properties of different photocatalysts that mentioned later. As shown in Figure 5.2(a), there are four sets of MB solutions tested. One set was put outside the black box and protected from light. The other set was put inside the box and protected from light, so there will be no effect from light but influences from heat induced by

illumination. The other two sets were both placed inside the box with illuminations from UV or visible lights respectively.

Figure 5.2 Schematic diagram (a) and absorbance decomposition kinetics (b) of MB at reference conditions.

One can see from Figure 5.2(b) that there is almost no degradation of MB at conditions with no light, under heat or illuminated with UV light. However, there are some spontaneous decompositions of MB noticed under visible light, which could be induced by MB photo-absorption. So, this self photo-induced reaction needs to be considered in the investigation of photocatalytic properties for TiO₂ nanotubes. The decomposition kinetics of MB under UV and visible light with no catalysts are used as references in all the following studies.

5.3.2 Photocatalytic properties of various single-walled TiO₂ nanotube

There are three set of TiO₂ nanotube samples fabricated and studied for their photocatalytic activity in MB aqueous solutions. DEG-HF NT, DMSO-HF NT and EG-NH₄F NT were prepared at 60 V with respective electrolytes. With different electrolyte compositions, tube morphologies vary.

Figure 5.3 shows the low magnification SEM images of obtained titanium oxide samples. The thicknesses of the films are similar and have range of 5 – 8 μm . DEG-HF nanotubes (Figure 5.2(a)) are highly ordered with diameter of 300 – 350 nm. DMSO-HF nanotubes (Figure 5.3(b)) are bundled in small areas with diameter of 200 – 300 nm. Furthermore, the tube tops are helical due to over etching. EG-NH₄F NT (Figure 5.3(c)) consists of closely packed tubes with diameter of 100 – 150 nm.

Figure 5.3 SEM images of DEG-HF NT (a), DMSO-HF NT (b) and DEG-NH₄F NT (c).

Figure 5.4 shows the Raman spectrums of samples before and after. There are no obvious peaks for TiO₂ samples before annealing, which suggests amorphous form. After annealing, peaks appear at 144, 399, 516 and 640 cm^{-1} which indicates the successful transformation to anatase phase [172]. And anatase is the form needed to perform photocatalytic activities.

Figure 5.4 Raman spectrums of nanotubes before and after annealing.

The elemental analysis of samples surfaces are presented in Table 5.1. Besides titanium and oxide, carbon and fluoride were also detected. It is obvious that the impurity atoms were incorporated in titanium dioxide from the electrolyte during the electrochemical process. The concentrations of the impurity atoms are up to a few percentages. The carbon concentration of DEG-HF NT (2.33 %) is smaller than that from DMSO-HF (3.86 %). And EG-NH₄F NT has the highest carbon concentration, which reaches up to 4.81 %.

Table 5.1 EDX analysis of samples

Element \ at. %	DEG-HF NT	DMSO-HF NT	EG-NH ₄ F NT
Ti	26.53	18.37	22.60
O	64.53	63.68	69.32
F	6.59	14.10	3.27
C	2.35	3.86	4.81

One EG-NH₄F NT sample was examined for concentration of chemical elements over depth. The Auger profile is displayed in Figure 5.5. The film thickness that sputtered is approximately one micrometer. Concentrations of titanium and oxide identify the stoichiometry of TiO₂ over the thickness. Concentration of carbon atoms is approximately uniform (5 %) across the thickness. The constant value suggests the penetration of impurity atoms into the oxide film by the electric field applied. A slight increase on the surface was noticed which could be due to the absorption of carbon dioxide from air. Since the impurities are built in from electrolyte, the elemental concentration is higher at the tube-electrolyte interface.

Figure 5.5 Depth concentration profiles of chemical elements for EG- NH_4F NT.

Figure 5.6 depicts decomposition kinetics of MB for TiO_2 nanotube samples under UV and visible irradiation. With UV irradiation (Figure 5.6 (a)), MB has almost no degradation after 54 hours illumination without any photocatalyst. The efficiencies with photocatalyst are all higher than 80 %, which are significantly more than that of the control solution and indicate the excellent photocatalytic properties of TiO_2 nanotubes. Titanium dioxide has band gap of 3.1 - 3.2 eV for anatase phase which acts actively at range of UV light [130]. The degradation efficiency was 82.4 % for DEG-HF NT, 95.3 % for DMSO-HF NT and 99.6 % for EG- NH_4F NT. The efficiency is related to tube diameters tightly, the smaller the diameter the higher the efficiency. For a fixed area, a smaller diameter means more tubes on samples hence larger surface area. The faster decomposition on MB for samples with larger surface area speaks of the influences of surface area on titanium dioxide's photocatalytic properties.

Figure 5.6 Decomposition profiles of MB for TiO₂ nanotube samples. (a) process under UV irradiation, (b) process under visible irradiation, (c) first and second usage of EG-NH₄F NT under visible light. (Sample size 2 cm², MB 8×10^{-6} g/L, no purge gas)

Unlike the case under UV irradiation, MB degraded by 43.3 % under visible light illumination without any photocatalyst (Figure 5.6(b)). The degradation was improved by 20 % with TiO₂ photocatalyst, even though the efficiency was not improved as significant as that under UV illumination. The enrichment in rapid decomposition with nanostructured titanium dioxide upon exposure to visible range could be brought by the impurities embedded from electrolyte. It is known that the carbon reduces the bandgap of TiO₂ to 2.7 – 2.8 eV by forming sublevels within [231]. The applied visible light has minimum wavelength of 425 nm, which requires bandgap of 2.9 eV for absorption. This number is reasonable since it lies in between of carbon-incorporated TiO₂ (2.7 –

2.8 eV) and pure TiO₂ material (3.1 – 3.2 eV). So, with the carbon embedded TiO₂, decomposition efficiency increases to 52 % for DEG-HF NT and increases further to 54.7% for DMSO NT. The efficiency for EG-NH₄F NT is the highest which reaches 62.1 %. This further proves the influences from surface area. Furthermore, it can be noticed that the increase in efficiency also agreed with the increase in carbon content from EDX analysis.

After the first usage under visible irradiation, EG-NH₄F NT was reused for the second time immediately with fresh MB solutions under visible light again. The result is shown in Figure 5.6(c). The degradation reduced from 62.1 % to 46.4 % compared to the 43.4 % of that from control solution after 54 hours. The drop could be due to the absorption of MB onto TiO₂ surface which slows down the degradation of MB from solution for the second usage.

5.3.3 Photocatalytic properties of double-walled TiO₂ nanotube

Double-walled TiO₂ nanotubes were fabricated with the same method as discussed in Section 3.3.3. The photocatalytic property of double-walled tubular structure are studied and presented here. A single-walled nanotube fabricated from same electrolyte with similar length was used for comparison.

Figure 5.7 (a) shows an example of double-walled TiO₂ nanotube structures prepared at 75 V with comparison to a single-walled structure in the insert. A clear inner wall and outer wall could be noticed. The outer tube diameter is around 150 - 200 nm. Duplicate samples were prepared and annealed at two different temperatures: 450 °C and 600 °C respectively. Figure 5.7 presents the TEM images of both samples. Since the forming mechanism of nanotube is

chemical etching and anatase is transformed with annealing, the fabricated anatase TiO₂ nanotubes are polycrystalline. And the final double-walled structure is formed mainly by mechanical strain during annealing so there is no significant gap between inner shell and outer shell noticed from the low resolution TEM images. However, big difference for double-walled TiO₂ NT annealed at 450 °C and 600 °C could still be noticed. Double-walled NT annealed at 450 °C has relatively smoother walls, which suggests the polycrystalline grains are closely packed with no obvious gap. On the other hand, the nanotube annealed at 600 °C has rough walls with cracks, which could be induced by mechanical strains at high temperature. It could be deduced from the wall roughness that the double-walled TiO₂ NT annealed at higher temperature has higher surface area hence should provide higher decomposition efficiency in water purification. The physical surface area of double-walled TiO₂ NT is 100 - 400 cm²/cm³ while the same parameter for single-walled TiO₂ NT is 20 - 40 cm²/cm³ according to BET analysis.

Figure 5.7 SEM image of double-walled structure (a) with insert of single-walled tube and TEM images of double-walled TiO₂ annealed at 450 °C (b, c) and 600° C (d, e).

The Raman and EDX results are described in Figure 5.8. Both samples annealed at 450 °C and 600 °C have anatase Raman peaks. Nanotubes annealed at 600 °C obviously have highly peak intensity than that annealed at 450 °C under the same laser power. This indicated that there are higher content of anatase phase in the doubled-walled sample annealed at 600 °C and not all contents are transferred to anatase under annealing at 450 °C. Furthermore, the carbon impurities embedded in nanotube could still be observed from Raman spectrum. As mentioned in Chapter 3 for the fabrication of double-walled TiO₂ nanotube, carbon was reduced almost half after annealing at 450 °C. Here, carbon peaks at 1360 and 1560 cm⁻¹ could still be observed clearly from the zoomed in spectrum especially for sample annealed at 450 °C. The peak intensity of carbon significantly decreased for nanotube annealed at 600 °C, which suggests further release of carbon for higher temperature annealing. This reduction on impurities was further verified from EDX analysis (table in Figure 5.8). The atomic percentage of carbon was further reduced from 3.3 % to 2.1 %.

Figure 5.8 Raman and EDX analysis of double-walled TiO₂ NTs annealed at 450 °C and 600 °C.

Figure 5.9 (a) depicts decomposition kinetics of MB for double-walled TiO₂ nanotube samples under UV irradiation. A single-walled TiO₂ nanotube fabricated from same electrolyte under similar conditions was also tested together for comparison. Data from MB solution without any photocatalyst were also draw as a control. For easier description, the double-walled TiO₂ nanotubes annealed at 450 °C and 600 °C are named double-walled 450 NT and double-walled 600 NT respectively. Under UV irradiation, the decomposition efficiency of double-walled 450 NT was 28.2 % after 54 hours, which was significant improvement compared to control solution (almost no degradation). The efficiency increased over 1.5 times to 45.8% for double-walled 600 NT. The main reason for this increase could be the expansion of surface area due to the cracked tube walls discussed earlier. Anyhow, both double-walled nanotubes have higher efficiency compared to single-walled sample with the same area whose degradation rate was only 24. 1%.

Figure 5.9 Decomposition profiles of MB for double-walled TiO₂ NTs under UV irradiation (a, b) and visible irradiation (c). (Sample size 1 cm², MB 8×10^{-6} g/L, no purge gas)

After the first experiment, the double-walled samples were taken out and put into newly made MB solution with initial concentration for the second process under UV irradiation. The results are showed in Figure 5.9 (b). As expected, efficiency drops. Both efficiencies drop by almost half of that for the first usage. Furthermore, the second usages of double-walled nanotube are less efficient compared to single-walled nanotube for the first usage.

The samples were taken out from the MB solution after the second usage and left in air for several days before the photocatalytic test under visible irradiation. These few days are meant for the MB residues from previous experiment to degrade. The decomposition kinetics are shown in Figure 5.9(c).

The degraded efficiency is 43.3 % for control solution, 46.4 % for single-walled NT, 51.0% for double-walled 450 NT and 59.5 % for double-walled 600 NT. The efficiency is increased as much as 40 %, which is almost double compared to the improvement from various single-walled NTs discussed in Section 5.3.2. Besides impurity atoms (carbon), this significant enrichment could also be contributed from the rich oxygen. It can be seen from the EDX analysis in Figure 5.8, the percentage of oxygen is 3.5 times of the titanium content. While in TiO₂, the ratio should be 2. The excess oxygen could come from the excellent adsorption property of double-walled structure compared to single-wall.

5.4 Summary

In this chapter, the photocatalytic properties of various single-walled and double-walled TiO₂ nanotube structures were investigated. In general, tubular structures provide much larger surface area hence excellent decomposition efficiency for MB. Under both UV and visible irradiation, nanotubes with smallest diameters demonstrate highest efficiency. The impurity atoms such as carbon that are incorporated into titanium dioxide during anodization pose positive influences on improving photocatalytic activity under visible illumination. Moreover, double-walled TiO₂ nanotubes show excellent degradation efficiency by providing more reaction sites from not only the double-walled structure but also the fractured wall induced from high temperature annealing. The excess oxygen absorption could also promote the enhancement in photocatalytic activity. In summary, this study suggests that

TiO₂ nanotubes, especially double-walled TiO₂ nanotubes, could be excellent photocatalysts for applications in clean energy.

Chapter 6 Fabrication of 3D TiO₂-Carbon Composites for Photocatalysis Application

6.1 Introduction

The photoelectronic property makes titanium dioxide excellent material in energy and environmental applications. As discussed in Chapter 5, nanotubes in particular have demonstrated better efficiency based on the enlarged surface area. Despite the good photocatalytic activity, there are still challenges. The recombination rate is a persistent phenomenon for titanium dioxide materials, which significantly decreases the decomposition efficiency [140]. Another challenge is the light absorption region. As titanium dioxide has relatively large bandgap (3.2 eV), the working range is in the UV region which is only a small portion from sun light [130]. Plenty of techniques have been tried to tackle the challenges and increase its photo-efficiency, by reducing the electron-hole recombination or red-shifting the photo-response to visible light region. These methods include doping with noble metal ions, non-metal ions and coupling with material whose bandgap is narrower [232-238].

The recombination of TiO₂ with carbon has been paid special attention. First of all, there are various carbon forms existing such as active carbon, carbon nanotube, graphite, graphene, amorphous carbon and so on. This makes it possible to produce plenty of TiO₂ and carbon composites. Moreover, carbon materials can act as ideal places for physisorption of contaminants [239-241]. Carbon materials could enhance the photocatalytic property of TiO₂ in several ways [160]: Firstly, carbon could act as sink for electrons which could enhance

the lifetime of the created charges. Secondly, carbon could create energy states in bandgap as surface impurity dopant. Furthermore, the defect sites of Ti-C or Ti-O-C could be formed which may enable absorption of visible lights [242]. There are many TiO₂-carbon composites that have been studied such as TiO₂ composited with graphene [243-245] and mixture of TiO₂ and CNTs [246-249]. Most of the composites fabricated show enhancement on photoactivity. However, most of the prepared composites are films made from commercially bought titanium dioxide or carbon materials.

In this chapter, we shall present the fabrication of several 3D TiO₂-carbon composites and investigate on their photoactivity for water purification. The composites prepared include TiO₂ nanotube with secondary CNTs, CNT/CNT network with TiO₂ coating and 3D graphite/graphene with TiO₂ coating.

6.2 Experimental

Fabrication of TiO₂ NT with secondary CNT

The CNTs here are called secondary CNTs as they are grown on the surface of other vertical aligned nanotubes (here are TiO₂ nanotubes). Nickel nitrate (Ni(NO₃)₂) solution in acetone with concentration of 0.01 M was used as catalyst precursor. A dose of 10 μ L was used on a surface of 1 cm $\times$ 1 cm. The actual growth happened in TCVD. After purging with high flow of Ar for five to ten minutes, tube temperature was ramped up to 700 °C at gas flow of 70 sccm H₂ and 200 sccm Ar. Ethanol vapor was used as carbon source and introduced into the TCVD system through a bubbler once the temperature reached 700 °C. The total growth time was controlled at 15 minutes after which

the carbon source was cut off and system was naturally cooled down. The growth mechanism will be discussed further in the discussion section later.

TiO₂ nanotubes were fabricated in DEG-HF electrolyte at 60 V for 72 hours. The as prepared samples were then annealed at 450 °C. One nanotube sample with catalyst precursor and one without were put in tubes for secondary CNT growth at 700 °C. Another pair of samples went through the same secondary CNT growth process at slightly lower temperature of 600 – 650 °C.

Fabrication of CNT / CNT network with TiO₂ coating

Primary vertical aligned CNTs were fabricated with 15 nm of nickel catalyst on silicon wafer. PECVD (Aixtron black magic) system was used for the fabrication with C₂H₂ as the carbon source. Growth was carried on for 10 minutes at 800 °C. Secondary CNTs were grown on the primary CNTs to form CNT network samples. Growth of secondary CNTs followed the same procedure as introduced above.

The Ni catalysts were removed by immersing CNT samples in acid solution for 5 – 10 minutes (HNO₃:H₂SO₄ = 1:3, diluted in water). CNT and CNT network samples were then immersed in water for a few minutes followed by immersion in IPA for another few minutes. TiO₂ coatings were formed with wet chemical method. The CNT samples were put in a mixture of IPA (25 ml, Regent Chemical) and titanium butoxide (Ti(OC(CH₃)₃)₄, Ti(OR)₄, 5 ml, Sigma-Aldrich) which was already stirred for half an hour. With continuous stirring, deionized water of 1 ml was added drop by drop. After thirty minutes, the mixture together with sample were sealed in autoclave and put in oven for

eight hours at 180 °C. Samples were washed thoroughly with ethanol and water for several times and dried naturally in air.

Samples were annealed at 450 °C in Ar atmosphere for one hour to stabilize the anatase titanium dioxide coating. At the same time, this heating process could also transfer any amorphous titania to anatase phase. Duplicates were annealed in 600 °C in air for two hours to fully remove the CNT / CNT network and leave only the TiO₂ structures. During the annealing process, carbon reacts with oxygen and form carbon dioxide which can be released from sample, therefore CNTs can be removed from samples. CNT and CNT network of the same size without any modifications were prepared as reference.

Fabrication of Graphite / graphene with TiO₂ coating

TCVD was used for the fabrication of graphite / graphene base. Ni foam cut into size of 2 cm × 7 cm was used as both the substrate and catalyst for the growth. Two strips of Ni foam were usually used for one process. The system was purged with high flow of Ar. The temperature was ramped up to 1000 °C in 15 minutes with protection of Ar (50 sccm) and H₂ (10 sccm). Once the temperature reached 1000 °C, gas flow was redirected to a bubbler before entering the reaction tube. Ethanol vapor was introduced through the bubbler and brought into tube as carbon source. The total growth time was controlled at 30 minutes after which the carbon source was cut off and tubes were naturally cooled down. The fabricated samples were cut into size of 1 cm × 1 cm and then coated with TiO₂ through the wet chemical method that introduced from above. Ni foam were cut into the same size and coated with TiO₂ without

carbon composite. Ni foam and graphite samples without any titanium dioxide coatings were also tested for photocatalytic properties as control samples.

Characterization

SEM was used to study the morphology of the structures fabricated. Raman was used to examine material phase. EDX was used to study the elemental composition.

Photocatalysis test

The black box was used for the investigation of photocatalytic properties of TiO₂ – carbon composite. Both UV and visible lights were used as illumination sources. All sample size used was 1 cm × 1 cm. 60 ml MB solution with concentration of 8×10^{-4} g/L was prepared for each sample. There were no stirring or purge gas used in the process. MB solution of 5 ml were taken out at time intervals of 0, 6, 24, 30, 48, 54 hours and stored with protection from light before next use. Absorbance of solutions was measured with Shimadzu UV-2450 spectrophotometer.

6.3 Result and Discussion

This section presents the fabrication of various TiO₂ – C composites and their application on photocatalysis.

6.3.1 Secondary CNT growth

For the growth of secondary CNTs, nickel nitrate in acetone was used as catalyst precursor. Acetone could evaporate after being dropped on substrate surface hence introduced no extra pollution from solvent. Moreover, acetone

has low polarity, which makes the solution easier to penetrate through the primary nanotubes (TiO₂ nanotube or primary CNT) instead of sitting on top [250]. As a result, nickel nitrate could be dispersed through primary nanotubes. Due to surface tension and primary sample's wettability, it is also possible that there is more precursor solution on the top half of the primary tubes compared to the bottom half that nearer to sample substrate. In experiment, the nickel nitrate in acetone was observed quickly absorbed by primary nanotube structure upon applying.

Figure 6.1 demonstrates the growth mechanism of secondary CNT. Figure 6.1(a, b) presents primary nanotube with nickel nitrate penetrated through. At high temperature, nickel nitrate decomposes and reduces to nickel particles (Figure 6.1(c)) by reactions as following:

Ethanol introduced into the tube would decompose at high temperature too and provide carbon source which is needed for secondary CNT growth. When carbon atoms saturate at catalyst particles, CNTs would be formed (Figure 6.1(d)). Ni catalysts are found at the tips of secondary CNTs, which means catalysts are lift up and remain at the tips [251]. So it could be hypothesized that the surface of the primary nanotube, either TiO₂ or CNT, is the base of secondary CNTs. Hence, carbon from secondary CNT is in direct contact with the TiO₂ surface or the primary CNT surface. This further suggests the formation of Ti-C or Ti-O-C in TiO₂ nanotube - CNT composites.

Figure 6.1 Growth mechanism of secondary CNT

The fabricated secondary CNTs usually have higher density at the surface of primary nanotubes and lower density at bottom. The reason is simple because there is more carbon source at surroundings and it is not easy for carbon source to penetrate through primary nanotube structures. Moreover, in our experiments, 700 °C is the standard temperature that is used for secondary CNT growth.

6.3.2 TiO₂ NT-CNT composite

There are two sets of TiO₂ NT – CNT composites fabricated. Both titania nanotubes fabricated from DEG-HF electrolyte at 60 V followed by one hour annealing at 450 °C (TiO₂ NT1 and TiO₂ NT2). TiO₂ NT1 was grown with secondary CNTs at lower temperature around 650 °C (TiO₂ NT1–CNT 650). During the growth, a duplicate of TiO₂ NT1 without catalyst was put aside and went through the same annealing process (TiO₂ NT1–650). The same processes were taken for TiO₂ NT2 at 700 °C and produced sample with (TiO₂ NT2-CNT 700) and without secondary CNTs (TiO₂ NT2-700).

The morphologies were studied using SEM, as shown in Figure 6.2. Both prepared TiO₂ nanotubes have diameters around 250 – 350 nm with lengths of 5 μm (Figure 6.2(a,b)). The annealing process at 450 °C introduced no change on

tube morphology. For both nanotube samples, after annealing at high temperature (Figure 6.2(c,d)), nanotube structures were maintained with fractures and cracks formed on tube walls which could extend the surface area. After growth of secondary CNTs at 650 °C, obvious thin CNT wires could be observed both on top and in between of the TiO₂ nanotubes (Figure 6.2(e)) for TiO₂ NT1. The grown CNTs are wire-like and have very small diameters compared to primary nanotubes which indicate the small size of Ni particles induced from nickel nitrate. From the cross section, it could be noticed that there are more secondary CNTs on top rather than on bottom (Figure 6.2(g)). The density of fabricated secondary CNTs is much higher at 700 °C as shown in Figure 6.2(f) for TiO₂ NT2. The thick secondary CNTs cover not only tube tops but also side of tubes as shown in the cross section image (Figure 6.3(h)). By comparing the two samples, it can be seen that temperature is essential for growth of secondary CNTs. Lower temperature (600 – 650 °C) produces secondary CNTs with less density. Furthermore, on the sample edge of TiO₂ NT2 – CNT 700, where there are no TiO₂ nanotubes, the growth of straight CNTs with larger diameter is noticed. Catalyst particles are bigger here; hence CNTs produced have larger diameters.

Figure 6.2 SEM images of TiO₂ nanotubes and TiO₂ nanotube-CNT composites.

(a) TiO₂ NT1, (b) TiO₂ NT2, (c) TiO₂ NT1-650, (d) TiO₂ NT2-700, (e,g) TiO₂ NT1-CNT 650, (f,h) TiO₂ NT2-CNT 700.

Titanium dioxide and its composite with CNT were interrogated by Raman spectroscopy. The Raman spectra of TiO₂ NT and TiO₂ NT – CNT are illustrated in Figure 6.3. Characteristic peaks for anatase are at 144, 399, 516 and 640 cm⁻¹ [252]. These peaks appeared for all samples and confirmed that the anatase phase remained unchanged after annealing and fabrication of secondary CNTs. D and G bands of carbon at around 1360 cm⁻¹ and 1600 cm⁻¹ [174] are observed for TiO₂ NT1-650 and NT2-700. These two samples were annealed during the fabrication process of secondary CNT. Even though there was no catalyst, they were still surrounded by carbon source and carbon could be incorporated into the structures during the high temperature annealing. After the growth of secondary CNT, much higher and narrower D and G bands are noticed; this confirms the successful growth of multi-walled CNTs. The bands shift to right by a few wavelengths for TiO₂ NT – CNT composites, which may indicate the interaction of CNTs with TiO₂ nanotubes. This may suggest the formation of Ti-C or Ti-O-C in TiO₂ nanotube - CNT composites.

Figure 6.3 Raman spectra of TiO₂ NT and TiO₂ NT-CNT composites.

EDX analysis further confirms the increase of carbon contents. As shown in Table 6.1, carbon content increases after annealing at the side of the CNT growth process. For TiO₂ NT1, carbon increases from 4.2 at. % to 6.56 at. % . For TiO₂ NT2 which was heated at higher temperature, this number increases from 1.85 at. % to 6.32 at. %. The number is obviously higher than that in TiO₂ NT1 due to the higher annealing temperature. After growth of secondary CNT, carbon content is 39.38 at.% for TiO₂ NT1 and 56.70 at.% for NT2. The high percentage confirms the formation of CNTs. Furthermore, the higher percentage at fabrication temperature of 700 °C also suggests higher secondary CNT density, which is consistent with SEM images.

Surface wettability also changes due to CNT. As indicated in Table 6.1, both TiO₂ NTs has superhydrophilic properties without composition of CNTs. With secondary CNTs of smaller density, surfaces are less hydrophilic with contact angle increased from several degrees to 13.6 degrees. The contact angle enlarges further to 44.2 degrees for TiO₂ NT with dense secondary CNTs. Despite increase of contact angle, surfaces still remain hydrophilic with angles smaller than 90 degrees. The increase in contact angle could be due to the introduce of secondary CNTs as CNTs are mostly hydrophobic.

Table 6.1 EDX and contact angle measurement of TiO₂ nanotube-CNT samples.

	TiO ₂ NT1	TiO ₂ NT1 – 650	TiO ₂ NT1 – CNT 650	TiO ₂ NT2	TiO ₂ NT2 - 700	TiO ₂ NT2 – CNT 700
EDX, Carbon (at. %)	4.20	6.56	39.38	1.83	6.32	56.70
Contact angle (°)	4.90	6.90	13.60	6.00	4.90	44.20

Figure 6.4 demonstrates the schematic diagram of photocatalytic activity of TiO₂ NT-CNT composite. Electrons are excited to CB under light irradiation,

creating electron-hole pairs. Typically, most of the pairs recombine very fast and only less than 1 % participates in photoactivities, which resulted in low efficiency [156]. The bandgap of TiO₂ is 3.2 eV and CB position is about – 4.21 eV using vacuum level as reference [156, 253] . Work functions of CNT ranges from- 4.5 eV to -5.0 eV which are lower than CB of TiO₂ [156, 254]. Since secondary CNT is based on and in contact with TiO₂ nanotube surface, electrons would flow from TiO₂ to CNT (process b in Figure 6.4), permitting charge separation and reducing recombination. The longer life of charges allows higher photoactivity hence higher efficiency. Furthermore, the aromatic regions of CNT could have strong stacking interaction with the aromatic regions of MB molecules (process a in Figure 6.4), which could provide high and faster affinity and adsorption of MB to CNT. This could promote photoactivity by drawing MB nearer to TiO₂ NT surfaces.

Figure 6.4 Schematic diagram of photocatalytic activity of TiO₂ NT-CNT composite. (a) adsorptivity of MB on CNT, (b) charge flow from TiO₂ to CNT

The photo-degradations of MB were evaluated with different photocatalysts and the results are shown in Figure 6.5. MB solution without any photocatalyst was used as control solution. Under UV irradiation, MB had no degradation

after 120 hours. Figure 6.5(a) indicates the degradation of MB under UV light for TiO₂ NT1 samples. The decomposition percentage is 34.8 % for TiO₂ NT1; this is significantly more than the control solution which indicates the photocatalytic activity of nanotubes. After annealing at 650 °C, the sample exhibits higher photocatalytic property with efficiency increases to 70.2 % which could be due to the enlarged surface area induced from higher temperature annealing. TiO₂ NT1 – CNT 650 shows evident improvement, achieving a decomposition percentage of 76.5 %. This is 9.3 % improvement compared to titanium dioxide nanotubes annealed at the same temperature. The enhanced photoactivity results from the excellent physisorption of carbon material and the hinder of charge recombination induced by CNT as electron acceptor. Similar trend is found for TiO₂ NT2 samples (Figure 6.5(b)). Efficiency increases from 37.5 % to 63.3 % as the annealing temperature rises. The degradation is enhanced to 83.8 % after compositing with secondary CNTs. It is noticed that the decomposition gained more than 30 % improvement. By comparing the enhancement on degradation of MB for both TiO₂ nanotube and CNT composite samples, it can be seen that the improvement is three times for sample with thicker secondary CNT than that with CNTs of less density. This further proves the enhancement on photocatalytic activity introduced by secondary CNTs.

Figure 6.5 (c,d) shows the result of MB decolorization under visible light irradiation in the presence of TiO₂ NT and NT-CNT composites. As shown in figures, the degradation efficiencies of MB are higher for all solutions (over 75 %) with photocatalyst than that without any (66 %). The incorporated carbon

impurities could be the possible explanation for this. Furthermore, all NT-CNT composites exhibit higher photoactivity than NT samples. The improvement by NT-CNT composites are relatively small compared to that under UV illumination. For TiO₂ NT1 with thin CNT, degradation of MB is only enhanced by 1 %. This percentage increases to 7 % for NT2 with denser secondary CNTs. The increase of photoactivity with more content of CNTs suggests that CNT acts as photosensitizer which could provide utilization of visible light in photocatalysis.

Figure 6.5 Decomposition efficiency of MB for TiO₂ NT-CNT composites. (a, b) under UV irradiation, (c, d) under visible illumination. (Sample size 1 cm², MB 8×10^{-6} g/L, no purge gas)

6.3.3 CNT - TiO₂ composite

Besides TiO₂ NT - CNT, composites of CNT with TiO₂ coatings were also fabricated and investigated for their photocatalytic properties. Both CNT and CNT network (primary CNT with secondary CNT) were used as substrate or template for TiO₂ coating. The CNT network was fabricated using the same batch of CNT samples. After titanium dioxide coating with wet chemical method, the samples were annealed at 450 °C to stabilize the oxide coating. Duplicates were produced by annealing further at 600 °C in air to fully remove any carbon material and leave titanium dioxide materials only.

TiO₂ was coated through the wet chemical method - solvothermal with hydrolysis of titanium butoxide. CNTs were immersed in solution and coated by titanium butoxide with continuous stirring. After adding in water, it went through a hydrolysis process and titanium oxide was produced. The overall reactions contain hydrolysis and condensation where condensation could happen as an oxolation [255, 256]. The Schematics could be as follows:

SEM images of CNT before and after TiO₂ coatings are shown in Figure 6.6. The as fabricated CNTs are vertically aligned and highly ordered (Figure 6.6(a)). After coating with TiO₂, the aligned CNTs demonstrate a pyramid-like microstructure (Figure 6.6(b)). CNTs are bundled together with small areas of several to 100 μm² with their tips closely packed but roots still attached to

substrate. After annealing at 450 °C (Figure 6.6(c)), the structures are still retained. The formation of pyramid-like structures could be due to the capillary forces [257, 258] . During the drying process, menisci are formed between adjacent nanotubes from which pressure difference between air and solvent is formed due to surface tension. This capillary interaction is the force that drives the formation of bundles as CNTs tend to bend in order to balance the capillary. Formation of more uniformly bundled CNTs has been reported with PSS solution [259].

Further annealing at 600 °C in air was taken to remove CNTs in the sample by oxidizing carbon to carbon dioxide. This sample was fabricated as a comparison for photocatalytic property study without influences from carbon. The detailed SEM (Figure 6.6(d)) shows that the pyramid-like structure is still maintained with porous tube walls (insert). The porous tube wall indicates that TiO₂ coatings on CNTs are not uniform, and it is possible that titanium dioxide covers not all the surface but leaves certain percentage of CNTs exposed. EDX was taken to examine the carbon content. The carbon was significantly reduced from 67.17 at.% to 11.15 at.%, indicating elimination of most CNTs in composite.

Figure 6.6 SEM images of aligned CNT (a); low resolution of CNT coated with TiO₂ (b); CNT – TiO₂ coating annealed at 450 °C (c) and TiO₂ sample after removing CNT template (d). Contact angle of CNT (e), CNT-TiO₂ coating (f) and TiO₂ – CNT template (g).

CNTs are superhydrophobic with contact angel of 130 degrees (Figure 6.6(e)). After coating with titanium dioxide, surface was changed to hydrophilic (52 degrees as in Figure 6.6(f)) because of the influence of hydrophilic titanium dioxide. It is also possible that certain surface energy is released by the pyramid-like structures, which resulted in the hydrophilic property. After remove of CNT, a superhydrophilic surface is achieved with contact angle of less than 10 degrees (Figure 6.6(g)). As mentioned earlier, titanium dioxide may not cover all of CNTs' walls so there are certain of carbon exposed. After high temperature annealing these parts of carbons were removed hence left only

titanium dioxide. However, the micro-structure is not altered before and after the annealing process. A hydrophilic surface is usually preferred than hydrophobic surface as hydrophilic surface could attract solutions which is helpful to photocatalytic activity. The surface wettability has influences especially at the beginning of process. It is easier for hydrophilic sample to enter MB solutions than hydrophobic sample; hydrophobic samples tend to float on the surface of solution and need to be pushed down into the solution. After the hydrophobic sample is immersed in solutions or irradiated with UV light, the surface usually turns into hydrophilic soon [260, 261].

Figure 6.7 shows SEM images of CNT network, CNT network coated with TiO₂ and TiO₂ structure after annealing with CNT network removed. Thick secondary CNTs can be observed on the primary vertically aligned CNTs (Figure 6.7 (a,b)). After TiO₂ coating, there are no pyramid-like structures formed for CNT network (Figure 6.7(c,d)) as the secondary CNTs provide extra support so that the primary CNTs would not tilt. Since the secondary CNTs are very thick and titanium butoxide in IPA solutions has certain viscosity, the solution was coated more on the secondary CNTs and stick together hence formed a thick film on top.

Duplicate of CNT network-TiO₂ composites was put under annealing at 600 °C in air to remove carbon materials (Figure 6.7 (e,f)). After annealing the height was reduced from 9 μm to 5 μm (Figure 6.7 (c,e)). This suggests collapse of the structure. It was possible a top-heavy structure is formed as most of titanium dioxides stick on the top. The bottom TiO₂ is not strong enough to hold the heavy head after removing of the supportive CNTs. EDX result

indicates reduction of carbon content from 70.3 at. % to 10.4 at.%. CNT network has similar wettability with CNTs and the wettability modification follows a similar trend: superhydrophobic to hydrophilic then superhydrophilic.

Figure 6.7 SEM image of CNT network (a,b), CNT network with TiO₂ coating (c,d), and TiO₂ coating with CNT network removed after annealing(e,f)

Raman spectroscopy was used to examine the material phase. Results are shown in Figure 6.8. Anatase has characteristic peaks at 144, 399, 516 and 640 cm⁻¹ and the peaks appears for all TiO₂-carbon composites before and after high temperature annealing. The appearance of anatase peaks before annealing

indicates formation of anatase directly after solvothermal process. D and G bands of carbon were observed for both CNT/CNT network – TiO₂ composites but disappeared after annealing at 600 °C. This result is consistent with EDX analysis and indicates the removal of carbon material.

Figure 6.8 Raman spectra of CNT/CNT network with TiO₂ coating.

Photocatalytic activities of samples were investigated by MB decolorization under both UV and visible light irradiation. The experimental efficiencies are illustrated in Figure 6.9. Figure 6.9 (a) and (b) indicate decolorization efficiency of MB with various photocatalysts. CNT / CNT network are not photocatalysts, but certain decolorization of MB could still be noticed because of their physisorption properties. CNTs adsorb certain amount of MB at the first six hours and the percentage maintains around 85 % for the following process.

This indicates adsorption saturation of CNTs. On the other hand, CNT network keeps adsorbing MB during the entire process with a faster decolorization rate at beginning and slower rate later. Compared to CNT sample, CNT network has much larger surface area due to the dense secondary CNTs therefore more adsorption potential for contaminants. For CNT-TiO₂ sample, decolorization efficiency is improved compared to TiO₂ sample. The decolorization rate is faster at the beginning but almost the same after 120 hours (Figure 6.9(a)). The improvement is not that significant. After the removal of CNTs, the fabricated TiO₂ nanotubes may have larger surface area as there is no blocking from CNTs at inside of walls. Hence the advantage from carbon composite could be counteracted by the enlarged surface area.

On the other hand, situation is quite different for CNT network-TiO₂ composite. Decolorization of MB from TiO₂ sample (18.9 %) is even smaller than the decolorization from CNT networks (39.6 %, Figure 6.9(b)). The compact TiO₂ film with collapse has smaller surface area hence lower photocatalytic efficiency. But it is glad to be noticed that CNT network – TiO₂ composites showed decomposition efficiency (44.7%) that is 2.5 time higher than TiO₂ sample. The advantages of carbon composites are fully proved here. Furthermore, the efficiency is enhanced by 25 % compared with the decolorization from CNT network. This gives more evidence of CNT network – TiO₂ composite as better photocatalyst. With secondary CNTs, CNT network provides more sites for TiO₂ coating therefore larger surface area. Furthermore, CNT network also provides more physisorption of MB compared to CNT sample.

Figure 6.9 (c) and (d) depict decomposition kinetics of MB for CNT/CNT network – TiO₂ composites. In both cases, TiO₂ samples could not beat CNT samples. The decomposition efficiencies of both TiO₂ samples are slightly lower than the decomposition efficiencies of carbon samples. CNTs could act as photosensitizers that adsorb visible light. Together with their physisorption of MB, CNTs enhance the self-degradation of MB. The CNT/CNT network – TiO₂ composite shows improvement on decomposition efficiency by a few percentages, which further proves the advantages of composites on photocatalytic activity.

Figure 6.9 Degradation of MB for CNT/CNT network – TiO₂ samples under UV (a,b) and visible light (c,d) irradiation. (Sample size 1 cm², MB 8×10^{-6} g/L, no purge gas)

6.3.4 Graphite/Graphene - TiO₂ composite

Besides CNT, carbon in graphite / graphene forms is also fabricated and composited with titanium dioxide. 3D Ni foams with porosity of 96 % were used both as substrate and catalyst. It is expected that the 3D structure provides larger surface area which could be good for photocatalytic activity. Ni foams coated with TiO₂ were fabricated as reference samples.

SEM images of Ni foam, Ni – TiO₂, graphite, graphite – TiO₂ composites are depicted in Figure 6.10. There is not much alteration on surface structures for Ni foam after coating with graphite / graphene (Figure 6.10 (a,d)). Titanium dioxide were coated both on Ni foam and graphite samples by solvothermal method followed with annealing at 450 °C. The surface morphologies of TiO₂ films on Ni foam before (Figure 6.10(b)) and after (Figure 6.10(c)) heating are quite similar. The hairy-like layers are titanium dioxide. After annealing, the smooth surface became fragmentary for graphite- TiO₂ sample (Figure 6.10 (e,f)). The spaces between fragments are bigger for areas with thin titania coating compared to thick coating (insert in (e, f)). The fragmentary structures could come from change on surface tension during annealing. Ni substrate is solid and stable so there are no cracks. The graphite and Titania coatings tend to shrink in different degrees hence resulted fragments. One good thing about fragmentary coatings is the enlarged surface area which can produce more sites for photoactivity. Similar to CNT and TiO₂ composite, graphite and Ni foam surfaces are hydrophobic (130°) and are tailed to superhydrophilic after solvothermal coating and annealing.

Figure 6.10 SEM images of: Ni foam (a), with TiO₂ coating before (b) and after (c) annealing; graphite/graphene (d), graphite – TiO₂ composite with (e) and without (f) annealing.

Figure 6.11 shows Raman spectra of graphite/graphene and various photocatalysts. Ni foam has no obvious peaks. The fabricated carbon materials on Ni foams contain both graphite and graphene. Typical bands of graphite and graphene could be found at 1600 and 2700 cm⁻¹, corresponding to G and 2D peak. Graphite has higher G peak compared to 2D peak. On the other hand, graphene has 2D peak with relatively higher intensity and a left shift to 2650 cm⁻¹ [176]. Most areas of the fabricated graphite sample have typical peaks of graphite. The D peak which indicates defects is very low for both graphite and graphene areas, indicating small defect percentage. Characteristic peaks of anatase appear at low wavenumbers in spectra for titania coating on both Ni foam and graphite substrates even before annealing. Raman spectra show that anatase retained with annealing. In the case of annealing, D peak of carbon appeared for Ni - TiO₂ and graphite-TiO₂ composites. More defects were introduced for graphite and the small red shift of the G peak suggests strong interaction between graphite and TiO₂. As the Ni-TiO₂ and graphite-TiO₂

samples were annealed together, amorphous carbon could be brought into Ni-TiO₂ composite from graphite by the gas flow.

Figure 6.11 Raman spectra of graphite / graphene with TiO₂ composites. * indicates the appearance of extra carbon peak.

Figure 6.12(a) depicts decolorization kinetics of MB for different photocatalysts with UV irradiation. Bare Ni foam and graphite substrate were tested too as control samples. Under UV illumination there was almost no decomposition of MB with substrate samples. Moreover, compared to Ni foam, graphite substrate demonstrated no difference in degradation efficiency, suggesting inconspicuous or no adsorption of MB. For photocatalysts, it is obvious that annealed samples demonstrated faster degradation rates for both sets. As observed from SEM images, the fragments induced by annealing could provide larger surface area. Furthermore, the as prepared titanium dioxides may

still have certain amount of amorphous materials which also transfer to anatase after heating. As result, both quantity and surface area of anatase are increased, enhancing the photocatalytic activities. The degradation efficiency is doubled to 26 % from 13 % for composite photocatalyst with graphite. This proves that combination with graphite / graphene is also a good choice to promote photocatalytic property of titanium dioxide.

Figure 6.12 Decolorization of MB with various graphite-TiO₂ composite under UV irradiation (a) and relative efficiency to Ni substrate under visible irradiation (b).

(Sample size 1 cm², MB 8 × 10⁻⁶ g/L, no purge gas)

The degradation kinetic of MB under visible irradiation is shown in Figure 6.12(b) with relative to the result from Ni foam. Unlike the case with UV, the

improvement on decomposition rate for the first 40 hours is not obvious with photocatalysts. Enhancements only start to show upon long time process (20 % improvement). Advantages of graphite – TiO₂ composite compared to Ni – TiO₂ is also not that significant. Even though, small improvement with a few percent was still noticed. It could be concluded that graphite – TiO₂ composites enhances photocatalytic efficiency better under UV irradiation.

6.4 Summary

In this chapter, we investigated various carbon and titanium dioxide composites for their photocatalytic properties. In general, carbon may act as ideal places for physisorption of contaminants, can act as photosensitizer to absorb visible light and conduct photoinduced charges to reduce the recombination rates. All these characteristics of carbon enhance the photocatalytic activities of titanium dioxide. Various 3D titanium dioxide and carbon composites were fabricated such as TiO₂ nanotube with CNT, CNT / CNT network with titania coating and graphite / graphene with titania coating. All the fabricated composites showed improvements on photocatalytic efficiencies especially under UV irradiation. In summary, this work suggests that titanium dioxide composited with carbon could be excellent photocatalysts.

Chapter 7 Conclusions and Future Work

7.1 Conclusions

In conclusion, titanium dioxide nanotubes with various morphologies have been fabricated by electrochemical anodization in organic electrolytes. The prepared nanotube structures have been investigated in both bone morphogenetic protein delivery and photocatalytic degradation of methylene blue.

Various TiO₂ nanotube structures have been successfully fabricated by anodization method. Experimental parameters, such as electrolyte, anodic voltage, experimental time and repeat usage of electrolyte, can all be used to tailor the tubular structures. Additionally, TiO₂ nanotubes with large diameter over 350 nm have been fabricated successfully for the first time by repeat use of DEG-HF electrolyte at high supplied potential. Tube diameter of 550 nm and 680 nm has been achieved at anodization voltage of 90 V and 100 V respectively. Finally, double-walled titania tubular structures have been successfully fabricated by low temperature anodization followed with subsequent annealing (in EG-NH₄F electrolyte). The low temperature can provide tube with thick walls and low porosity. The annealing process split the tubes into two concentric tubes. The mainly reason for splitting could be the mechanical radial strains, introduced by release of impurity atoms and volume shrinking during annealing process.

As a popular implant material in bone graft, TiO_2 nanotubes with different outlooks, diameters lengths and phases have shown their influences on protein delivery. In general, Titania nanotubes provide super hydrophilic surface and more loading volume for proteins. Tube diameter and density has obvious effect on protein elution. Wider diameter and large gaps allow ease of entering and eluting of solvent from nanotubes. The elution is most profoundly affected by tube length. Samples with longer tubes provide more loading sites and allow deeper penetration. The change of material phase doesn't affect surface wettabilities but does introduce some structure change which provides minor influences on the overall elution.

Titanium dioxide is also well known as a popular photocatalyst. Both various single-walled and double-walled TiO_2 nanotube structures have shown good photocatalytic property for methylene blue decomposition under both UV and white light irradiation. Overall, tubular structures provide much larger surface area hence excellent decomposition efficiency for MB. The impurity atoms, incorporated into titanium dioxide during anodization, pose positive influences on improving photocatalytic activity under visible illumination. Furthermore, the fabricated double-walled TiO_2 nanotubes have demonstrated excellent degradation efficiency as compared to single-walled structure prepared from the same electrolyte. The double-walled structure has the potential to provide more reaction sites and excess oxygen absorption.

To further improve the photocatalytic property of titanium dioxide, various titanium dioxide and carbon composite materials has been fabricated. Carbon materials could act as ideal places for physisorption of contaminants.

Furthermore, carbon can act as photosensitizer to absorb visible light and conduct photoinduced charges away to reduce the recombination rates. The fabricated 3D titanium dioxide and carbon composites have shown their positive effect on improving photocatalytic efficiencies. TiO₂ nanotube with secondary CNT, CNT/CNT network with titania coating and graphite / graphene with titania coating have all demonstrated enhancement on photocatalytic activities especially under UV irradiation.

7.2 Future Work Recommendations

7.2.1 TiO₂ nanotube structure for BMP delivery

As shown in Chapter four, BMP-2 elution could be affected by different titanium dioxide nanotube aspects, such as diameter, length and phases. It is obvious that delivery of BMP-2 could be controlled by TiO₂ nanotube structures with different morphologies. This study suggests that the TiO₂ nanotube structures can be used as a suitable carrier for BMP-2 release for enhanced bone repair and regeneration. Currently studies on protein delivery are processed in vitro. Current investigation focus on titanium dioxide material only, it will be interesting to study composite carrier mixed by titanium dioxide and polymers. The future research in this direction would be in vivo study of BMP-2 delivery with TiO₂ carriers. Bioactivity of proteins and cell adhesions could both be investigated in vivo for delivery systems based on titanium dioxide nanotubes.

The study on BMP delivery with TiO₂ nanotube structures could be useful for actual osteoblast operations. TiO₂ nanotube coatings could be introduced

easily to any Ti implant surface in any shape, large or small size, such as dental implant, hip joint prostheses and stents. With the combination with BMPs, TiO₂ nanotube surface coating could provide reinforcement of implant, stronger osteoblast adhesion and better cellular interactions.

7.2.2 TiO₂ nanotube structure for photocatalysis

The photocatalytic properties of titanium dioxide are studied for water purification under both UV and white light irradiation. The results are showed in Chapter five and six. The enhanced photocatalytic efficiencies, especially under UV irradiation, suggest that TiO₂ nanotubes, especially double-walled TiO₂ nanotubes, and TiO₂-Carbon composite could be excellent photocatalysts for applications in clean energy, including water purification, air purification, self cleaning and solar cells. The current focus on TiO₂-Carbon composites are based on CNT and graphite/graphene. The future research in this direction could focus on fabrication of composites with other carbon forms such as amorphous carbon and aligned carbon.

Titanium dioxide, its nanotube and composite can be used widely in various environmental applications, including sterilization, air purification, water purification, defogger, self-cleaning, solar cells or even hydrogen generations from water splitting. The enhanced photocatalytic efficiencies of TiO₂ nanotube and its carbon composites could enhance sterilization and pollution management effects.

List of Publications

Journal papers:

1. **Liang, K.**, Tay, B.K., Kupreeva, O.V., Orekhovskaya, T.I., Lazarouk, S.K., and Borisenko, V.E., *Fabrication of Double-walled titania nanotubes and Their photocatalytic Activity*. ACS Sustainable Chem.Eng., 2014. **2**(4): p. 991-995.
2. **Liang, K.**, Li, X.C. and Tay, B.K., *Study of bone Morphogenetic Protein-2 Delivery with Different TiO₂ Nanotube Structures*, Nanoscience and Nanotechnology Letters, 2013. **5**(2): p.162-166.
3. **Liang, K.**, Li, X.C. and Tay, B.K., *Fabrication of Large Diameter TiO₂ Nanotube for Bone Morphogenetic Protein-2 Delivery*, International Journal of Nanotechnology, 2013. Accepted, in press.
4. Kupreeva, O.V., Lazarouk, S.K., Borisenko, V.E., **Liang, K.** and Tay, B.K., *Photocatalytic Properties of Nanostructured Titanium Dioxide*, Journal of Applied Spectroscopy, 2013. **79**(6): p. 1010-1013.
5. Liu, W.W., Lu, C.X., **Liang, K.**, Tay, B.K., *A three dimensional vertically aligned multiwall carbon nanotube/NiCo₂O₄ core/shell structure for novel high-performance supercapacitors*, Journal of Materials Chemistry A, 2014. **2**: p. 5100-5107.
6. Li, X.C., **Liang, K.**, Tay, B.K., Teo, Edwin H., *Morphology-tunable Assembly of Periodically Aligned Si Nanowire and Radial pn Junction Arrays for Solar Cell Applications*, Applied Surface Science, 2012. **258**(17): p.6169-6176.

7. Li, X.C., Tay, B.K., Li, J.S., Tan, D., Tan, C.W., **Liang, K.**, *Mildly Reduced Graphene Oxide-Ag Nanoparticles Hybrid film for Surface Enhanced Raman Scattering*, Nanoscale Research Letters, 2012. **7**:205, p. 1-8.

Conference Papers:

1. **Liang, K.**, Li, X.C. and Tay, B.K., *Large Diameter TiO₂ Nanotube Fabrication for Bone Morphogenetic Protein Delivery*, Nanoelectronics Conference (INEC), IEEE 4th International, 2011. Digital Object Identifier: 10.1109/INEC.2011.5991774. Tao Yuan, Taiwan.
2. **Liang, K.**, Li, X.C and Tay, B.K., *Bone Morphogenetic Protein-2 Delivery with TiO₂ Nanotube Structures*, Sino-Singapore Symposium on Functional Nanomaterials and Bioelectronics, 2011. Nanjing, China.
3. **Liang, K.**, Li, X.C., and Tay, B.K., *Characterization of TiO₂ Nanotube Fabricated with Anodization Method Using Dimethyl Sulfoxide Electrolytes*, 9th Joint Symposium on Material Science and Engineering for the 21st Century, 2009. Kyoto, Japan.
4. Kupreeva, O.V., Borisenko, V.E., Kardynal, B., Grutzmacher, D., **Liang, K.**, and Tay, B.K., *Double-walled Titania Nanotubes*, Physics, Chemistry and Application of Nanostructures: Proceedings of International Conference Nanomeeting-2013, 2013. p.351-354.
5. Li, X.C., Loh, G.C., **Liang, K.**, Tay, B.K., Baillargeat, D., *Heat Conduction across Multiwalled Carbon Nanotube/Graphene Hybrid Films*, The 13th Electronics Packaging Technology Conference (EPTC), 2011. Singapore.

Bibliography

1. Berger, H., H. Tang, and F. Lévy, *Growth and Raman spectroscopic characterization of TiO₂ anatase single crystals*. Journal of Crystal Growth, 1993. **130**(1–2): p. 108-112.
2. Maile, F.J., G. Pfaff, and P. Reynders, *Effect pigments—past, present and future*. Progress in Organic Coatings, 2005. **54**(3): p. 150-163.
3. Braun, J.H., A. Baidins, and R.E. Marganski, *TiO₂ pigment technology: a review*. Progress in Organic Coatings, 1992. **20**(2): p. 105-138.
4. Fujishima, A. and K. Honda, *Electrochemical Photolysis of Water at a Semiconductor Electrode*. Nature, 1972. **238**(5358): p. 37-38.
5. Fujishima, A., T.N. Rao, and D.A. Tryk, *Titanium dioxide photocatalysis*. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 2000. **1**(1): p. 1-21.
6. Gratzel, M., *Photoelectrochemical cells*. Nature, 2001. **414**(6861): p. 338-344.
7. Hagfeldt, A. and M. Graetzel, *Light-Induced Redox Reactions in Nanocrystalline Systems*. Chemical Reviews, 1995. **95**(1): p. 49-68.
8. Gerischer, H., *Photocatalysis in aqueous solution with small TiO₂ particles and the dependence of the quantum yield on particle size and light intensity*. Electrochimica Acta, 1995. **40**(10): p. 1277-1281.
9. Mills, A. and S. Le Hunte, *An overview of semiconductor photocatalysis*. Journal of Photochemistry and Photobiology A: Chemistry, 1997. **108**(1): p. 1-35.
10. Qiu, J., et al., *Composite Titanium dental implant fabricated by electro-discharge compaction*. Biomaterials, 1997. **18**(2): p. 153-160.
11. Drnovšek, N., et al., *The properties of bioactive TiO₂ coatings on Ti-based implants*. Surface and Coatings Technology, 2012. **209**(0): p. 177-183.
12. Li, Y., et al., *Glucose biosensor based on the room-temperature phosphorescence of TiO₂/SiO₂ nanocomposite*. Biosensors and Bioelectronics, 2009. **24**(12): p. 3706-3710.
13. Signoretto, M., et al., *Effect of textural properties on the drug delivery behaviour of nanoporous TiO₂ matrices*. Microporous and Mesoporous Materials, 2011. **139**(1–3): p. 189-196.
14. Alivisatos, A.P., *Semiconductor Clusters, Nanocrystals, and Quantum Dots*. Science, 1996. **271**(5251): p. 933-937.
15. Yin, Y. and A.P. Alivisatos, *Colloidal nanocrystal synthesis and the organic-inorganic interface*. Nature, 2005. **437**(7059): p. 664-670.
16. Chen, X., et al., *Doped Semiconductor Nanomaterials*. Journal of Nanoscience and Nanotechnology, 2005. **5**(9): p. 1408-1420.
17. Bayati, M.R., F. Golestani-Fard, and A.Z. Moshfegh, *Visible photodecomposition of methylene blue over micro arc oxidized WO₃-loaded TiO₂ nano-porous layers*. Applied Catalysis A: General, 2010. **382**(2): p. 322-331.
18. Kobayashi, N., et al., *Comparative pulmonary toxicity study of nano-TiO₂ particles of different sizes and agglomerations in rats: Different*

- short- and long-term post-instillation results. Toxicology*, 2009. **264**(1–2): p. 110-118.
19. Park, J.-A., et al., *Structural, electrical and gas sensing properties of eletrospun TiO₂ nanofibers*. *Thin Solid Films*, 2010. **518**(22): p. 6642-6645.
20. Zhang, X., et al., *Fabrication and photocatalytic activity of porous TiO₂ nanowire microspheres by surfactant-mediated spray drying process*. *Materials Research Bulletin*, 2009. **44**(5): p. 1070-1076.
21. Mor, G.K., et al., *Use of Highly-Ordered TiO₂ Nanotube Arrays in Dye-Sensitized Solar Cells*. *Nano Letters*, 2005. **6**(2): p. 215-218.
22. Dong, X., et al., *Oriented TiO₂ nanowire array grown on curved surface of Ti wire with superior photoelectrochemical properties*. *Applied Surface Science*, 2013. **270**(0): p. 457-461.
23. Choi, H., E. Stathatos, and D.D. Dionysiou, *Sol–gel preparation of mesoporous photocatalytic TiO₂ films and TiO₂/Al₂O₃ composite membranes for environmental applications*. *Applied Catalysis B: Environmental*, 2006. **63**(1–2): p. 60-67.
24. Attar, A.S., et al., *Sol–gel template synthesis and characterization of aligned anatase-TiO₂ nanorod arrays with different diameter*. *Materials Chemistry and Physics*, 2009. **113**(2–3): p. 856-860.
25. Zeng, X., et al., *Amphiphilic and photocatalytic behaviors of TiO₂ nanotube arrays on Ti prepared via electrochemical oxidation*. *Journal of Alloys and Compounds*, 2011. **509**(24): p. L221-L227.
26. Roy, S.C., M. Paulose, and C.A. Grimes, *The effect of TiO₂ nanotubes in the enhancement of blood clotting for the control of hemorrhage*. *Biomaterials*, 2007. **28**(31): p. 4667-4672.
27. Popat, K.C., et al., *Titania Nanotubes: A Novel Platform for Drug-Eluting Coatings for Medical Implants?* *Small*, 2007. **3**(11): p. 1878-1881.
28. Popat, K.C., et al., *Decreased Staphylococcus epidermis adhesion and increased osteoblast functionality on antibiotic-loaded titania nanotubes*. *Biomaterials*, 2007. **28**(32): p. 4880-4888.
29. Paulose, M., et al., *TiO₂ Nanotube Arrays of 1000 μ m Length by Anodization of Titanium Foil: Phenol Red Diffusion*. *The Journal of Physical Chemistry C*, 2007. **111**(41): p. 14992-14997.
30. Paulose, M., et al., *Fabrication of mechanically robust, large area, polycrystalline nanotubular/porous TiO₂ membranes*. *Journal of Membrane Science*, 2008. **319**(1–2): p. 199-205.
31. Kruss, S., et al., *Carbon nanotubes as optical biomedical sensors*. *Advanced Drug Delivery Reviews*, 2013. **65**(15): p. 1933-1950.
32. Wong, B.S., et al., *Carbon nanotubes for delivery of small molecule drugs*. *Advanced Drug Delivery Reviews*, 2013. **65**(15): p. 1964-2015.
33. Hopley, E.L., et al., *Carbon nanotubes leading the way forward in new generation 3D tissue engineering*. *Biotechnology Advances*, (0).
34. Puleo, D.A., et al., *Osteoblast responses to orthopedic implant materials in vitro*. *Journal of Biomedical Materials Research*, 1991. **25**(6): p. 711-723.

35. Tengvall, P. and I. Lundström, *Physico-chemical considerations of titanium as a biomaterial*. Clinical Materials, 1992. **9**(2): p. 115-134.
36. Lanone, S., et al., *Determinants of carbon nanotube toxicity*. Advanced Drug Delivery Reviews, 2013. **65**(15): p. 2063-2069.
37. Bussy, C., L. Methven, and K. Kostarelos, *Hemotoxicity of carbon nanotubes*. Advanced Drug Delivery Reviews, 2013. **65**(15): p. 2127-2134.
38. Bhattacharya, K., et al., *Mechanisms of carbon nanotube-induced toxicity: Focus on pulmonary inflammation*. Advanced Drug Delivery Reviews, 2013. **65**(15): p. 2087-2097.
39. Bella, F. and R. Bongiovanni, *Photoinduced polymerization: An innovative, powerful and environmentally friendly technique for the preparation of polymer electrolytes for dye-sensitized solar cells*. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 2013. **16**(0): p. 1-21.
40. Kazuhito hashimoto, H.i.a.A.F., *TiO₂ Photocatalysis: A Historical Overview and Future Prospects*. Japanese Journal of Applied Physics, 2005. **44**(12): p. 8269-8285.
41. Nakata, K., et al., *Photoenergy conversion with TiO₂ photocatalysis: New materials and recent applications*. Electrochimica Acta, 2012. **84**(0): p. 103-111.
42. Fujishima, A. and X. Zhang, *Titanium dioxide photocatalysis: present situation and future approaches*. Comptes Rendus Chimie, 2006. **9**(5): p. 750-760.
43. Akira Fujishima, K.H., Toshiya Watanabe, *TiO₂ Photocatalysis: Fundamentals and Applications*. 1999: Tokyo Bkc.
44. Akira Fujishima, S.k., *Clean and Green: A Story of the Sun and Photocatalysts*. 2011: Kaisei-sya Tokyo.
45. Ollis, D., P. Pichat, and N. Serpone, *TiO₂ photocatalysis-25 years*. Applied Catalysis B: Environmental, 2010. **99**(3-4): p. 377.
46. D.Zhang, G.L., J.C. Yu, *Energy Efficiency and Renewable Energy Through nanotechnology*, ed. L. Zhang. 2011, London: Springer.
47. Yu, J.-G., et al., *Aqueous adsorption and removal of organic contaminants by carbon nanotubes*. Science of The Total Environment, 2014. **482-483**(0): p. 241-251.
48. Ren, X., et al., *Carbon nanotubes as adsorbents in environmental pollution management: A review*. Chemical Engineering Journal, 2011. **170**(2-3): p. 395-410.
49. Mattia, D., K.P. Lee, and F. Calabrò, *Water permeation in carbon nanotube membranes*. Current Opinion in Chemical Engineering, 2014. **4**(0): p. 32-37.
50. Bae, C., et al., *Template-Directed Synthesis of Oxide Nanotubes: Fabrication, Characterization, and Applications†*. Chemistry of Materials, 2008. **20**(3): p. 756-767.
51. Pang, Y.L., et al., *A critical review on the recent progress of synthesizing techniques and fabrication of TiO₂-based nanotubes photocatalysts*. Applied Catalysis A: General, 2014. **481**(0): p. 127-142.

52. Ou, H.-H. and S.-L. Lo, *Review of titania nanotubes synthesized via the hydrothermal treatment: Fabrication, modification, and application*. Separation and Purification Technology, 2007. **58**(1): p. 179-191.
53. Macak, J.M., et al., *TiO₂ nanotubes: Self-organized electrochemical formation, properties and applications*. Current Opinion in Solid State and Materials Science, 2007. **11**(1-2): p. 3-18.
54. Macak, J.M., et al., *Smooth Anodic TiO₂ Nanotubes*. Angewandte Chemie International Edition, 2005. **44**(45): p. 7463-7465.
55. Zwillling, V., et al., *Structure and physicochemistry of anodic oxide films on titanium and TA6V alloy*. Surface and Interface Analysis, 1999. **27**(7): p. 629-637.
56. Ghicov, A. and P. Schmuki, *Self-ordering electrochemistry: a review on growth and functionality of TiO₂ nanotubes and other self-aligned MOx structures*. Chemical Communications, 2009. **0**(20): p. 2791-2808.
57. Xie, Z.B. and D.J. Blackwood, *Effects of anodization parameters on the formation of titania nanotubes in ethylene glycol*. Electrochimica Acta, 2010. **56**(2): p. 905-912.
58. Bauer, S., S. Kleber, and P. Schmuki, *TiO₂ nanotubes: Tailoring the geometry in H₃PO₄/HF electrolytes*. Electrochemistry Communications, 2006. **8**(8): p. 1321-1325.
59. Kover, F. and J. Musselin, *A comparative study of anodic oxide films on titanium, niobium and tantalum*. Thin Solid Films, 1968. **2**(3): p. 211-234.
60. Aladjem, A., *Anodic oxidation of titanium and its alloys*. Journal of Materials Science, 1973. **8**(5): p. 688-704.
61. Omidvar, H., et al., *Influence of anodization parameters on the morphology of TiO₂ nanotube arrays*. Superlattices and Microstructures, 2011. **50**(1): p. 26-39.
62. Ghicov, A., et al., *Titanium oxide nanotubes prepared in phosphate electrolytes*. Electrochemistry Communications, 2005. **7**(5): p. 505-509.
63. Sreekantan, S., et al., *Influence of electrolyte pH on TiO₂ nanotube formation by Ti anodization*. Journal of Alloys and Compounds, 2009. **485**(1-2): p. 478-483.
64. Rani, S., et al., *Synthesis and applications of electrochemically self-assembled titania nanotube arrays*. Physical Chemistry Chemical Physics, 2010. **12**(12): p. 2780-2800.
65. G.K. Mor, O.K.V., Maggie Paulose, Niloy Mukherjee and Craig A. Grimes *Fabrication of Tapered, Conical-shaped Titania Nanotubes*. Journal of Materials Research, 2003. **18**: p. 2588-2593.
66. Balaur, E., et al., *Wetting behaviour of layers of TiO₂ nanotubes with different diameters*. Journal of Materials Chemistry, 2005. **15**(42): p. 4488-4491.
67. Zhao, J., et al., *Fabrication of titanium oxide nanotube arrays by anodic oxidation*. Solid State Communications, 2005. **134**(10): p. 705-710.
68. Qingyun Cai, M.P., Oomman K. Varghese and Craig A. Grimes . , *The Effect of Electrolyte Composition on the Fabrication of Self-Organized Titanium Oxide Nanotube Arrays by Anodic Oxidation*. Journal of Materials Research, 2005. **20**: p. 230-236.

69. L.V. Taveira, J.M.m., h.Tsuchiya, L.F.P.Dick and P.Schmuki, *Initiation and Growth of Self-Organized TiO₂ Nanotubes Anodically Formed in NH₄F/(NH₄)₂SO₄ Electrolytes*. Journal of The Electrochemical Society, 2005. **152**(10): p. B405-B410.
70. Yoriya, S., et al., *Initial Studies on the Hydrogen Gas Sensing Properties of Highly-Ordered High Aspect Ratio TiO₂ Nanotube-Arrays 20 m to 222 m in Length*. Sensor Letters, 2006. **4**(3): p. 334-339.
71. Su, Y. and Y. Deng, *Effect of structure on the photocatalytic activity of Pt-doped TiO₂ nanotubes*. Applied Surface Science, 2011. **257**(23): p. 9791-9795.
72. Prakasam, H.E., et al., *A New Benchmark for TiO₂ Nanotube Array Growth by Anodization*. The Journal of Physical Chemistry C, 2007. **111**(20): p. 7235-7241.
73. Allam, N.K. and C.A. Grimes, *Formation of Vertically Oriented TiO₂ Nanotube Arrays using a Fluoride Free HCl Aqueous Electrolyte*. J Phys Chem C Nanomater Interfaces, 2007. **111**(35): p. 13028-13032.
74. Allam, N.K., K. Shankar, and C.A. Grimes, *Photoelectrochemical and water photoelectrolysis properties of ordered TiO₂ nanotubes fabricated by Ti anodization in fluoride-free HCl electrolytes*. Journal of Materials Chemistry, 2008. **18**(20): p. 2341-2348.
75. Chen, X., et al., *Fabrication of 10 nm diameter TiO₂ nanotube arrays by titanium anodization*. Thin Solid Films, 2007. **515**(24): p. 8511-8514.
76. Frayret, J.P., R. Pointeau, and A. Caprani, *Anodic behaviour of titanium in acidic chloride containing media (HCl NaCl). Influence of the constituents of the medium — I. Study of the stationary current. Calculation of the overall reaction orders*. Electrochimica Acta, 1981. **26**(12): p. 1783-1788.
77. Urist, M.R., *Bone: Formation by Autoinduction*. Science, 1965. **150**(3698): p. 893-899.
78. Urist, M. and B. Strates, *The Classic: Bone Morphogenetic Protein*. Clinical Orthopaedics and Related Research®, 2009. **467**(12): p. 3051-3062.
79. A.J. Celeste, J.A.I., R.C. Taylor, R.M. Hewick, V.Rosen, E.A. Wang and J.M.Wozney, *Identification of Transforming Growth Factor Beta Family Members Present in bone-Inductive PRotein Purified from Bovine Bone*. Proc. Natl. Acad. Sci. USA., 1990. **87**(24): p. 9843-9847.
80. WOZNEY, J.M., et al., *Novel Regulators of Bone Formation: Molecular Clones and Activities*. Obstetrical & Gynecological Survey, 1989. **44**(5): p. 387.
81. Bessa, P.C., M. Casal, and R.L. Reis, *Bone morphogenetic proteins in tissue engineering: the road from the laboratory to the clinic, part I (basic concepts)*. Journal of Tissue Engineering and Regenerative Medicine, 2008. **2**(1): p. 1-13.
82. Hogan, B.L.M., *Bone morphogenetic proteins: Multifunctional regulators of vertebrate development*. Genes & Development, 1996. **10**(13): p. 1580-1594.

83. Axelrad, T.W. and T.A. Einhorn, *Bone morphogenetic proteins in orthopaedic surgery*. Cytokine & Growth Factor Reviews, 2009. **20**(5–6): p. 481-488.
84. Termaat, M.F., et al., *Bone Morphogenetic Proteins Development and Clinical Efficacy in the Treatment of Fractures and Bone Defects*. The Journal of Bone & Joint Surgery, 2005. **87**(6): p. 1367-1378.
85. Reddi, A.H., *Role of morphogenetic proteins in skeletal tissue engineering and regeneration*. Nat Biotech, 1998. **16**(3): p. 247-252.
86. Haidar, Z., R. Hamdy, and M. Tabrizian, *Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part A: Current challenges in BMP delivery*. Biotechnology Letters, 2009. **31**(12): p. 1817-1824.
87. Engstrand, T., et al., *A Novel Biodegradable Delivery System for Bone Morphogenetic Protein-2*. Plastic and Reconstructive Surgery, 2008. **121**(6): p. 1920-1928 10.1097/PRS.0b013e31817151b0.
88. Dard, M., et al., *Tools for tissue engineering of mineralized oral structures*. Clinical Oral Investigations, 2000. **4**(2): p. 126-129.
89. Hollinger, J.O., et al., *Recombinant human bone morphogenetic protein-2 and collagen for bone regeneration*. Journal of Biomedical Materials Research, 1998. **43**(4): p. 356-364.
90. Giannoudis, P.V., T.A. Einhorn, and D. Marsh, *Fracture healing: The diamond concept*. Injury, 2007. **38**, **Supplement 4**(0): p. S3-S6.
91. Haidar, Z., R. Hamdy, and M. Tabrizian, *Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part B: Delivery systems for BMPs in orthopaedic and craniofacial tissue engineering*. Biotechnology Letters, 2009. **31**(12): p. 1825-1835.
92. Li, R.H. and J.M. Wozney, *Delivering on the promise of bone morphogenetic proteins*. Trends in Biotechnology, 2001. **19**(7): p. 255-265.
93. Koyama, N., et al., *Experimental study of osteoinduction using a new material as a carrier for bone morphogenetic protein-2*. British Journal of Oral and Maxillofacial Surgery, 2011. **49**(4): p. 314-318.
94. Lee, K.Y. and S.H. Yuk, *Polymeric protein delivery systems*. Progress in Polymer Science, 2007. **32**(7): p. 669-697.
95. Kim, H.D. and R.F. Valentini, *Retention and activity of BMP-2 in hyaluronic acid-based scaffolds in vitro*. Journal of Biomedical Materials Research, 2002. **59**(3): p. 573-584.
96. Geiger, M., R.H. Li, and W. Friess, *Collagen sponges for bone regeneration with rhBMP-2*. Advanced Drug Delivery Reviews, 2003. **55**(12): p. 1613-1629.
97. Seeherman, H. and J.M. Wozney, *Delivery of bone morphogenetic proteins for orthopedic tissue regeneration*. Cytokine & Growth Factor Reviews, 2005. **16**(3): p. 329-345.
98. Schuessele, A., et al., *Enhanced bone morphogenetic protein-2 performance on hydroxyapatite ceramic surfaces*. Journal of Biomedical Materials Research Part A, 2009. **90A**(4): p. 959-971.

99. Johan Forsgren, B.U., Mihranyan Albert, Engqvist Hakan and Stromme Maria, *Fast Loading, Slow Release A New Strategy For Incorporating Antibiotics to Hydroxyapatite*. Bioceramics 21, 2009: p. 523-526.
100. Xie, G., et al., *Hydroxyapatite nanoparticles as a controlled-release carrier of BMP-2: absorption and release kinetics in vitro*. Journal of Materials Science: Materials in Medicine, 2010. **21**(6): p. 1875-1880.
101. Sohier, J., et al., *Porous beta tricalcium phosphate scaffolds used as a BMP-2 delivery system for bone tissue engineering*. Journal of Biomedical Materials Research Part A, 2010. **92A**(3): p. 1105-1114.
102. Lee, S.-H. and H. Shin, *Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering*. Advanced Drug Delivery Reviews, 2007. **59**(4-5): p. 339-359.
103. Majid, K., et al., *Biomimetic calcium phosphate coatings as bone morphogenetic protein delivery systems in spinal fusion*. The Spine Journal, 2011. **11**(6): p. 560-567.
104. Hu, Y., et al., *Development of a porous poly(L-lactic acid)/hydroxyapatite/collagen scaffold as a BMP delivery system and its use in healing canine segmental bone defect*. Journal of Biomedical Materials Research Part A, 2003. **67A**(2): p. 591-598.
105. Niu, X., et al., *Porous nano-HA/collagen/PLLA scaffold containing chitosan microspheres for controlled delivery of synthetic peptide derived from BMP-2*. Journal of Controlled Release, 2009. **134**(2): p. 111-117.
106. Luginbuehl, V., et al., *Localized delivery of growth factors for bone repair*. European Journal of Pharmaceutics and Biopharmaceutics, 2004. **58**(2): p. 197-208.
107. Haidar, Z.S., R.C. Hamdy, and M. Tabrizian, *Protein release kinetics for core-shell hybrid nanoparticles based on the layer-by-layer assembly of alginate and chitosan on liposomes*. Biomaterials, 2008. **29**(9): p. 1207-1215.
108. Yilgor, P., N. Hasirci, and V. Hasirci, *Sequential BMP-2/BMP-7 delivery from polyester nanocapsules*. Journal of Biomedical Materials Research Part A, 2010. **93A**(2): p. 528-536.
109. Engel, E., et al., *Nanotechnology in regenerative medicine: the materials side*. Trends in Biotechnology, 2008. **26**(1): p. 39-47.
110. Sandhiya, S., S.A. Dkhar, and A. Surendiran, *Emerging trends of nanomedicine – an overview*. Fundamental & Clinical Pharmacology, 2009. **23**(3): p. 263-269.
111. Nair, L.S. and C.T. Laurencin, *Nanofibers and Nanoparticles for Orthopaedic Surgery Applications*. The Journal of Bone & Joint Surgery, 2008. **90**(Supplement_1): p. 128-131.
112. Ochsenbein, A., et al., *Osteoblast responses to different oxide coatings produced by the sol-gel process on titanium substrates*. Acta Biomaterialia, 2008. **4**(5): p. 1506-1517.
113. Lord, M.S., M. Foss, and F. Besenbacher, *Influence of nanoscale surface topography on protein adsorption and cellular response*. Nano Today, 2010. **5**(1): p. 66-78.

114. Hori, N., et al., *Selective cell affinity of biomimetic micro-nano-hybrid structured TiO₂ overcomes the biological dilemma of osteoblasts*. Dental Materials, 2010. **26**(4): p. 275-287.
115. Chiang, C.-Y., et al., *Formation of TiO₂ nano-network on titanium surface increases the human cell growth*. Dental Materials, 2009. **25**(8): p. 1022-1029.
116. Demetrescu, I., C. Pirvu, and V. Mitran, *Effect of nano-topographical features of Ti/TiO₂ electrode surface on cell response and electrochemical stability in artificial saliva*. Bioelectrochemistry, 2010. **79**(1): p. 122-129.
117. So, K., et al., *Reinforcement of tendon attachment to bioactive porous titanium by BMP-2-induced ectopic bone formation*. Journal of Biomedical Materials Research Part A, 2010. **93A**(4): p. 1410-1416.
118. Bae, I.-H., et al., *Anodic oxidized nanotubular titanium implants enhance bone morphogenetic protein-2 delivery*. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2010. **93B**(2): p. 484-491.
119. Balasundaram, G., C. Yao, and T.J. Webster, *TiO₂ nanotubes functionalized with regions of bone morphogenetic protein-2 increases osteoblast adhesion*. Journal of Biomedical Materials Research Part A, 2008. **84A**(2): p. 447-453.
120. Brammer, K.S., C.J. Frandsen, and S. Jin, *TiO₂ nanotubes for bone regeneration*. Trends in Biotechnology, 2012. **30**(6): p. 315-322.
121. Frandsen, C.J., et al., *Hybrid micro/nano-topography of a TiO₂ nanotube-coated commercial zirconia femoral knee implant promotes bone cell adhesion in vitro*. Materials Science and Engineering: C, 2013. **33**(5): p. 2752-2756.
122. Terzian, R., et al., *Photocatalyzed mineralization of cresols in aqueous media with irradiated titania*. Journal of Catalysis, 1991. **128**(2): p. 352-365.
123. Linsebigler, A.L., G. Lu, and J.T. Yates, *Photocatalysis on TiO₂ Surfaces: Principles, Mechanisms, and Selected Results*. Chemical Reviews, 1995. **95**(3): p. 735-758.
124. Hoffmann, M.R., et al., *Environmental Applications of Semiconductor Photocatalysis*. Chemical Reviews, 1995. **95**(1): p. 69-96.
125. Rajeshwar, K., *Photoelectrochemistry and the environment*. Journal of Applied Electrochemistry, 1995. **25**(12): p. 1067-1082.
126. Zlamal, M., et al., *Electrochemically assisted photocatalysis on self-organized TiO₂ nanotubes*. Electrochemistry Communications, 2007. **9**(12): p. 2822-2826.
127. Fujishima, A., X. Zhang, and D.A. Tryk, *TiO₂ photocatalysis and related surface phenomena*. Surface Science Reports, 2008. **63**(12): p. 515-582.
128. Augugliaro, V., et al., *Overview on oxidation mechanisms of organic compounds by TiO₂ in heterogeneous photocatalysis*. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 2012. **13**(3): p. 224-245.

129. Nakata, K. and A. Fujishima, *TiO₂ photocatalysis: Design and applications*. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 2012. **13**(3): p. 169-189.
130. Huang, J., et al., *Band gap narrowing of TiO₂ by compensated codoping for enhanced photocatalytic activity*. Journal of Natural Gas Chemistry, 2012. **21**(3): p. 302-307.
131. Anpo, M., *Utilization of TiO₂ Photocatalysts in Green Chemistry*. Pure and Applied Chemistry, 2000. **72**(7): p. 1265-1270.
132. Zhu, J., et al., *Hydrothermal doping method for preparation of Cr³⁺-TiO₂ photocatalysts with concentration gradient distribution of Cr³⁺*. Applied Catalysis B: Environmental, 2006. **62**(3-4): p. 329-335.
133. Chen, H., et al., *Reaction of NH₃ with Titania: N-Doping of the Oxide and TiN Formation*. The Journal of Physical Chemistry C, 2006. **111**(3): p. 1366-1372.
134. Belver, C., et al., *Nitrogen-containing TiO₂ photocatalysts: Part 2. Photocatalytic behavior under sunlight excitation*. Applied Catalysis B: Environmental, 2006. **65**(3-4): p. 309-314.
135. Tada, H., et al., *All-solid-state Z-scheme in CdS-Au-TiO₂ three-component nanojunction system*. Nat Mater, 2006. **5**(10): p. 782-786.
136. Miyauchi, M., et al., *A Highly Hydrophilic Thin Film Under 1 μW/cm² UV Illumination*. Advanced Materials, 2000. **12**(24): p. 1923-1927.
137. Takeshita, K., et al., *Transient IR absorption study of charge carriers photogenerated in sulfur-doped TiO₂*. Journal of Photochemistry and Photobiology A: Chemistry, 2006. **177**(2-3): p. 269-275.
138. Hsu, S.-W., et al., *Ion-assisted electron-beam evaporation of carbon-doped titanium oxide films as visible-light photocatalyst*. Thin Solid Films, 2007. **515**(7-8): p. 3521-3526.
139. Li, D., et al., *Visible-Light-Driven N-F-Codoped TiO₂ Photocatalysts. 2. Optical Characterization, Photocatalysis, and Potential Application to Air Purification*. Chemistry of Materials, 2005. **17**(10): p. 2596-2602.
140. Zhang, L., et al., *Kinetics and mechanisms of charge transfer processes in photocatalytic systems: A review*. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 2012. **13**(4): p. 263-276.
141. Iwata, K., et al., *Carrier Dynamics in TiO₂ and Pt/TiO₂ Powders Observed by Femtosecond Time-Resolved Near-Infrared Spectroscopy at a Spectral Region of 0.9–1.5 μm with the Direct Absorption Method*. The Journal of Physical Chemistry B, 2004. **108**(52): p. 20233-20239.
142. Subramanian, V., E.E. Wolf, and P.V. Kamat, *Catalysis with TiO₂/Gold Nanocomposites. Effect of Metal Particle Size on the Fermi Level Equilibration*. Journal of the American Chemical Society, 2004. **126**(15): p. 4943-4950.
143. Silva, C.G. and J.L. Faria, *Photocatalytic Oxidation of Phenolic Compounds by Using a Carbon Nanotube-Titanium Dioxide Composite Catalyst*. ChemSusChem, 2010. **3**(5): p. 609-618.
144. Ou, Y., et al., *MWNT-TiO₂:Ni composite catalyst: A new class of catalyst for photocatalytic H₂ evolution from water under visible light illumination*. Chemical Physics Letters, 2006. **429**(1-3): p. 199-203.

145. Xiang, Q., J. Yu, and M. Jaroniec, *Graphene-based semiconductor photocatalysts*. Chemical Society Reviews, 2012. **41**(2): p. 782-796.
146. Hu, Z., et al., *Visible light driven photodynamic anticancer activity of graphene oxide/TiO₂ hybrid*. Carbon, 2012. **50**(3): p. 994-1004.
147. Tran, P.D., et al., *Recent advances in hybrid photocatalysts for solar fuel production*. Energy & Environmental Science, 2012. **5**(3): p. 5902-5918.
148. Chen, J.S., et al., *TiO₂ and SnO₂@TiO₂ hollow spheres assembled from anatase TiO₂ nanosheets with enhanced lithium storage properties*. Chemical Communications, 2010. **46**(43): p. 8252-8254.
149. Yun, H.J., et al., *Influence of Aspect Ratio of TiO₂ Nanorods on the Photocatalytic Decomposition of Formic Acid*. The Journal of Physical Chemistry C, 2009. **113**(8): p. 3050-3055.
150. Zhao, T., et al., *Multichannel TiO₂ hollow fibers with enhanced photocatalytic activity*. Journal of Materials Chemistry, 2010. **20**(24): p. 5095-5099.
151. Liang, H.-c. and X.-z. Li, *Effects of structure of anodic TiO₂ nanotube arrays on photocatalytic activity for the degradation of 2,3-dichlorophenol in aqueous solution*. Journal of Hazardous Materials, 2009. **162**(2-3): p. 1415-1422.
152. Liu, Z., et al., *Efficient Photocatalytic Degradation of Gaseous Acetaldehyde by Highly Ordered TiO₂ Nanotube Arrays*. Environmental Science & Technology, 2008. **42**(22): p. 8547-8551.
153. Chen, D., et al., *Carbon and Nitrogen Co-doped TiO₂ with Enhanced Visible-Light Photocatalytic Activity*. Industrial & Engineering Chemistry Research, 2007. **46**(9): p. 2741-2746.
154. Dong, F., et al., *Enhancement of the Visible Light Photocatalytic Activity of C-Doped TiO₂ Nanomaterials Prepared by a Green Synthetic Approach*. The Journal of Physical Chemistry C, 2011. **115**(27): p. 13285-13292.
155. Wang, D.-H., et al., *One-step hydrothermal synthesis of N-doped TiO₂/C nanocomposites with high visible light photocatalytic activity*. Nanoscale, 2012. **4**(2): p. 576-584.
156. Yao, Y., et al., *Photoreactive TiO₂/Carbon Nanotube Composites: Synthesis and Reactivity*. Environmental Science & Technology, 2008. **42**(13): p. 4952-4957.
157. Yu, H., et al., *TiO₂-carbon nanotube heterojunction arrays with a controllable thickness of TiO₂ layer and their first application in photocatalysis*. Journal of Photochemistry and Photobiology A: Chemistry, 2008. **200**(2-3): p. 301-306.
158. Zhou, K., et al., *Preparation of graphene-TiO₂ composites with enhanced photocatalytic activity*. New Journal of Chemistry, 2011. **35**(2): p. 353-359.
159. Liu, J., et al., *Gram-scale production of graphene oxide-TiO₂ nanorod composites: Towards high-activity photocatalytic materials*. Applied Catalysis B: Environmental, 2011. **106**(1-2): p. 76-82.
160. Leary, R. and A. Westwood, *Carbonaceous nanomaterials for the enhancement of TiO₂ photocatalysis*. Carbon, 2011. **49**(3): p. 741-772.

161. Kashi, M.A., et al., *Self-ordering of anodic nanoporous alumina fabricated by accelerated mild anodization method*. Thin Solid Films, 2010. **518**(23): p. 6767-6772.
162. Okada, M., et al., *Self-Organized Formation of Short TiO₂ Nanotube Arrays By Complete Anodization of Ti Thin Films*. Physics Procedia, 2012. **32**(0): p. 714-718.
163. Bauersfeld, M.L., P. Neumaier, and J. Wöllenstein, *Nanoporous Tungsten Trioxide Grown by Electrochemical Anodization of Tungsten for Gas Sensing Applications*. Procedia Engineering, 2012. **47**(0): p. 204-207.
164. Lee, S.-F., Y.-P. Chang, and L.-Y. Lee, *Synthesis of carbon nanotubes on silicon nanowires by thermal chemical vapor deposition*. New Carbon Materials, 2011. **26**(6): p. 401-407.
165. Vargas Garcia, J.R. and T. Goto, *Thermal barrier coatings produced by chemical vapor deposition*. Science and Technology of Advanced Materials, 2003. **4**(4): p. 397-402.
166. Bobrovnik, S.A., *Determination of antibody affinity by ELISA. Theory*. Journal of Biochemical and Biophysical Methods, 2003. **57**(3): p. 213-236.
167. Halim, N.D., A.W. Joseph, and B.K. Lipska, *A novel ELISA using PVDF microplates*. Journal of Neuroscience Methods, 2005. **143**(2): p. 163-168.
168. Toth, M., B.L. Thiel, and A.M. Donald, *Interpretation of secondary electron images obtained using a low vacuum SEM*. Ultramicroscopy, 2003. **94**(2): p. 71-87.
169. Sturm, H., et al., *SEM/EDX: Advanced investigation of structured fire residues and residue formation*. Polymer Testing, 2012. **31**(5): p. 606-619.
170. Andrews, D.L., *Rayleigh Scattering and Raman Effect, Theory*, in *Encyclopedia of Spectroscopy and Spectrometry (Second Edition)*, L. Editor-in-Chief: John, Editor. 1999, Academic Press: Oxford. p. 2413-2419.
171. Vishwas, M., K. Narasimha Rao, and R.P.S. Chakradhar, *Influence of annealing temperature on Raman and photoluminescence spectra of electron beam evaporated TiO₂ thin films*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2012. **99**(0): p. 33-36.
172. Xu, J.P., et al., *Effect of manganese ions concentration on the anatase-rutile phase transformation of TiO₂ films*. Journal of Physics and Chemistry of Solids, 2009. **70**(3-4): p. 511-515.
173. Sarkar, S. and P.K. Das, *Thermal and structural stability of single- and multi-walled carbon nanotubes up to 1800 °C in Argon studied by Raman spectroscopy and transmission electron microscopy*. Materials Research Bulletin, 2013. **48**(1): p. 41-47.
174. Vollebregt, S., et al., *Influence of the growth temperature on the first and second-order Raman band ratios and widths of carbon nanotubes and fibers*. Carbon, 2012. **50**(10): p. 3542-3554.

175. Ferrari, A.C., *Raman spectroscopy of graphene and graphite: Disorder, electron–phonon coupling, doping and nonadiabatic effects*. Solid State Communications, 2007. **143**(1–2): p. 47-57.
176. Ferrari, A.C., et al., *Raman Spectrum of Graphene and Graphene Layers*. Physical Review Letters, 2006. **97**(18): p. 187401.
177. Beatty, S.M. and J.E. Smith, *Fractional wettability and contact angle dynamics in burned water repellent soils*. Journal of Hydrology, 2010. **391**(1–2): p. 97-108.
178. L, et al., *UV–Vis spectroscopic and chemometric study on the aggregation of ionic dyes in water*. Talanta, 1999. **49**(1): p. 99-106.
179. Lee, B.-G., et al., *Formation behavior of anodic TiO₂ nanotubes in fluoride containing electrolytes*. Transactions of Nonferrous Metals Society of China, 2009. **19**(4): p. 842-845.
180. Tian, M., et al., *Significant enhancement of the photoelectrochemical activity of TiO₂ nanotubes*. Electrochemistry Communications, 2011. **13**(11): p. 1186-1189.
181. Sun, L., et al., *An electrochemical strategy of doping Fe³⁺ into TiO₂ nanotube array films for enhancement in photocatalytic activity*. Solar Energy Materials and Solar Cells, 2009. **93**(10): p. 1875-1880.
182. Kwon, Y., et al., *Enhanced ethanol sensing properties of TiO₂ nanotube sensors*. Sensors and Actuators B: Chemical, 2012. **173**(0): p. 441-446.
183. Şennik, E., et al., *Synthesis of highly-ordered TiO₂ nanotubes for a hydrogen sensor*. International Journal of Hydrogen Energy, 2010. **35**(9): p. 4420-4427.
184. Sánchez, M. and M.E. Rincón, *Sensor response of sol–gel multiwalled carbon nanotubes-TiO₂ composites deposited by screen-printing and dip-coating techniques*. Sensors and Actuators B: Chemical, 2009. **140**(1): p. 17-23.
185. Ni, J., et al., *Fabrication of thin film TiO₂ nanotube arrays on Co–28Cr–6Mo alloy by anodization*. Materials Science and Engineering: C, 2013. **33**(3): p. 1460-1466.
186. Brammer, K.S., et al., *Comparative cell behavior on carbon-coated TiO₂ nanotube surfaces for osteoblasts vs. osteo-progenitor cells*. Acta Biomaterialia, 2011. **7**(6): p. 2697-2703.
187. Park, H., et al., *Fabrication of dye-sensitized solar cells by transplanting highly ordered TiO₂ nanotube arrays*. Solar Energy Materials and Solar Cells, 2011. **95**(1): p. 184-189.
188. Lee, J. and J.Y. Jho, *Fabrication of highly ordered and vertically oriented TiO₂ nanotube arrays for ordered heterojunction polymer/inorganic hybrid solar cell*. Solar Energy Materials and Solar Cells, 2011. **95**(11): p. 3152-3156.
189. Chen, X. and S.S. Mao, *Titanium Dioxide Nanomaterials: Synthesis, Properties, Modifications, and Applications*. Chemical Reviews, 2007. **107**(7): p. 2891-2959.
190. Lu, Z., et al., *Study of effect annealing temperature on the structure, morphology and photocatalytic activity of Si doped TiO₂ thin films deposited by electron beam evaporation*. Applied Surface Science, 2011. **257**(24): p. 10715-10720.

191. Hamadani, M., A. Reisi-Vanani, and A. Majedi, *Synthesis, characterization and effect of calcination temperature on phase transformation and photocatalytic activity of Cu,S-codoped TiO₂ nanoparticles*. Applied Surface Science, 2010. **256**(6): p. 1837-1844.
192. Peng, L., et al., *Long-Term Small Molecule and Protein Elution from TiO₂ Nanotubes*. Nano Letters, 2009. **9**(5): p. 1932-1936.
193. Albu, S.P. and P. Schmuki, *Influence of anodization parameters on the expansion factor of TiO₂ nanotubes*. Electrochimica Acta, 2013. **91**(0): p. 90-95.
194. Yoriya, S., et al., *Fabrication of Vertically Oriented TiO₂ Nanotube Arrays Using Dimethyl Sulfoxide Electrolytes*. The Journal of Physical Chemistry C, 2007. **111**(37): p. 13770-13776.
195. Wang, J. and Z. Lin, *Anodic Formation of Ordered TiO₂ Nanotube Arrays: Effects of Electrolyte Temperature and Anodization Potential*. The Journal of Physical Chemistry C, 2009. **113**(10): p. 4026-4030.
196. Albu, S.P., et al., *Formation of Double-Walled TiO₂ Nanotubes and Robust Anatase Membranes*. Advanced Materials, 2008. **20**(21): p. 4135-4139.
197. Yu, L., et al., *BMP2 induces segment-specific skeletal regeneration from digit and limb amputations by establishing a new endochondral ossification center*. Developmental Biology, 2012. **372**(2): p. 263-273.
198. Chappuis, V., et al., *Periosteal BMP2 activity drives bone graft healing*. Bone, 2012. **51**(4): p. 800-809.
199. Vogt, S., et al., *The influence of the stable expression of BMP2 in fibrin clots on the remodelling and repair of osteochondral defects*. Biomaterials, 2009. **30**(12): p. 2385-2392.
200. Hernández, A., et al., *Material-related effects of BMP-2 delivery systems on bone regeneration*. Acta Biomaterialia, 2012. **8**(2): p. 781-791.
201. Yilgor, P., et al., *Incorporation of a sequential BMP-2/BMP-7 delivery system into chitosan-based scaffolds for bone tissue engineering*. Biomaterials, 2009. **30**(21): p. 3551-3559.
202. Lee, J.W., et al., *Bone regeneration using a microstereolithography-produced customized poly(propylene fumarate)/diethyl fumarate photopolymer 3D scaffold incorporating BMP-2 loaded PLGA microspheres*. Biomaterials, 2011. **32**(3): p. 744-752.
203. Nie, H. and C.-H. Wang, *Fabrication and characterization of PLGA/HAp composite scaffolds for delivery of BMP-2 plasmid DNA*. Journal of Controlled Release, 2007. **120**(1-2): p. 111-121.
204. Maire, M., et al., *Bovine BMP osteoinductive potential enhanced by functionalized dextran-derived hydrogels*. Biomaterials, 2005. **26**(24): p. 5085-5092.
205. Ginebra, M.-P., et al., *Calcium phosphate cements as drug delivery materials*. Advanced Drug Delivery Reviews, 2012. **64**(12): p. 1090-1110.
206. Lakhkar, N.J., et al., *Bone formation controlled by biologically relevant inorganic ions: Role and controlled delivery from phosphate-based glasses*. Advanced Drug Delivery Reviews, 2013. **65**(4): p. 405-420.

207. Boerckel, J.D., et al., *Effects of protein dose and delivery system on BMP-mediated bone regeneration*. Biomaterials, 2011. **32**(22): p. 5241-5251.
208. Hunziker, E.B., et al., *Osseointegration: The slow delivery of BMP-2 enhances osteoinductivity*. Bone, 2012. **51**(1): p. 98-106.
209. Kashiwagi, K., T. Tsuji, and K. Shiba, *Directional BMP-2 for functionalization of titanium surfaces*. Biomaterials, 2009. **30**(6): p. 1166-1175.
210. Zhao Jun Han, K.k.O., Cher Ming Tan, beng Kang Tay, Sean A.F. Peel, *Effect of Hydrophilicity of Carbon Nanotube Arrays on the Release Rate and Activity of Recombinant Human Bone Morphogenetic Protein-2*. Nanotechnology, 2011. **22**(29): p. 295712 -08.
211. L.M. Hocking, A.D.R., *The Spreading of a Drop by Capillary Action*. Journal of Fluid Mechanics, 1982. **121**: p. 425-442.
212. González-García, C., et al., *Effect of nanoscale topography on fibronectin adsorption, focal adhesion size and matrix organisation*. Colloids and Surfaces B: Biointerfaces, 2010. **77**(2): p. 181-190.
213. Zhang, J., et al., *Mechanism of enhanced photocatalysis of TiO₂ by Fe³⁺ in suspensions*. Applied Surface Science, 2011. **258**(4): p. 1294-1299.
214. Addamo, M., et al., *Oxidation of oxalate ion in aqueous suspensions of TiO₂ by photocatalysis and ozonation*. Catalysis Today, 2005. **107-108**(0): p. 612-618.
215. Choi, H., et al., *Chapter 8 TiO₂-Based Advanced Oxidation Nanotechnologies for Water Purification and Reuse*, in *Sustainability Science and Engineering*, C.E. Isabel and I.S. Andrea, Editors. 2010, Elsevier. p. 229-254.
216. Zhang, L., et al., *Development of TiO₂ photocatalyst reaction for water purification*. Separation and Purification Technology, 2003. **31**(1): p. 105-110.
217. Bai, H., et al., *Hierarchical 3D dendritic TiO₂ nanospheres building with ultralong 1D nanoribbon/wires for high performance concurrent photocatalytic membrane water purification*. Water Research, (0).
218. Chen, J., et al., *Theoretical studies on spectroscopic properties of ruthenium sensitizers absorbed to TiO₂ film surface with connection mode for DSSC*. Dyes and Pigments, 2012. **94**(3): p. 459-468.
219. Lee, Y., J. Chae, and M. Kang, *Comparison of the photovoltaic efficiency on DSSC for nanometer sized TiO₂ using a conventional sol-gel and solvothermal methods*. Journal of Industrial and Engineering Chemistry, 2010. **16**(4): p. 609-614.
220. Chae, J., et al., *Photovoltaic efficiency on dye-sensitized solar cells (DSSC) assembled using Ga-incorporated TiO₂ materials*. Journal of Industrial and Engineering Chemistry, 2010. **16**(6): p. 906-911.
221. Li, F., Q. Li, and H. Kim, *Spray deposition of electrospun TiO₂ nanoparticles with self-cleaning and transparent properties onto glass*. Applied Surface Science, 2013. **276**(0): p. 390-396.

222. Kesmez, Ö., et al., *Sol-gel preparation and characterization of anti-reflective and self-cleaning SiO₂-TiO₂ double-layer nanometric films*. Solar Energy Materials and Solar Cells, 2009. **93**(10): p. 1833-1839.
223. Madaeni, S.S. and N. Ghaemi, *Characterization of self-cleaning RO membranes coated with TiO₂ particles under UV irradiation*. Journal of Membrane Science, 2007. **303**(1-2): p. 221-233.
224. Li, F.-t., et al., *N-doped P25 TiO₂-amorphous Al₂O₃ composites: One-step solution combustion preparation and enhanced visible-light photocatalytic activity*. Journal of Hazardous Materials, 2012. **239-240**(0): p. 118-127.
225. Cantau, C., et al., *N-doped TiO₂ by low temperature synthesis: Stability, photo-reactivity and singlet oxygen formation in the visible range*. Journal of Photochemistry and Photobiology A: Chemistry, 2010. **216**(2-3): p. 201-208.
226. Chen, F., et al., *Photocatalytic performance of a visible light TiO₂ photocatalyst prepared by a surface chemical modification process*. Catalysis Communications, 2009. **10**(11): p. 1510-1513.
227. Kuo, Y.-L., et al., *A study of parameter setting and characterization of visible-light driven nitrogen-modified commercial TiO₂ photocatalysts*. Journal of Hazardous Materials, 2011. **190**(1-3): p. 938-944.
228. Ananpattarachai, J., P. Kajitvichyanukul, and S. Seraphin, *Visible light absorption ability and photocatalytic oxidation activity of various interstitial N-doped TiO₂ prepared from different nitrogen dopants*. Journal of Hazardous Materials, 2009. **168**(1): p. 253-261.
229. Huang, D., et al., *A new method to prepare high-surface-area N-TiO₂/activated carbon*. Materials Letters, 2011. **65**(2): p. 326-328.
230. Mohammadi, M.R., et al., *Preparation of high surface area titania (TiO₂) films and powders using particulate sol-gel route aided by polymeric fugitive agents*. Sensors and Actuators B: Chemical, 2006. **120**(1): p. 86-95.
231. Wu Z., D.F., Zhao W., Wang H., Liu Y., Guan B., *The Fabrication and Characterization of Novel Carbon Doped TiO₂ Nanotubes, Nanowires and Nanorods with High Visible Light Photocatalytic Activity*. nanotechnology, 2009. **20**(23): p. 235701.
232. Di Valentin, C. and G. Pacchioni, *Trends in non-metal doping of anatase TiO₂: B, C, N and F*. Catalysis Today, 2013. **206**(0): p. 12-18.
233. Aysin, B., A. Ozturk, and J. Park, *Silver-loaded TiO₂ powders prepared through mechanical ball milling*. Ceramics International, 2013. **39**(6): p. 7119-7126.
234. Song, G.B., et al., *Preparation and phase transformation of anatase-rutile crystals in metal doped TiO₂/muscovite nanocomposites*. Thin Solid Films, 2005. **491**(1-2): p. 110-116.
235. Luiz, D.d.B., et al., *Photocatalytic reduction of nitrate ions in water over metal-modified TiO₂*. Journal of Photochemistry and Photobiology A: Chemistry, 2012. **246**(0): p. 36-44.
236. Wen, Z. and L. Tian-mo, *Hydrogen sensing characteristics and mechanism of nanosize TiO₂ dope with metallic ions*. Physica B: Condensed Matter, 2010. **405**(2): p. 564-568.

237. Diker, H., et al., *Characterizations and photocatalytic activity comparisons of N-doped nc-TiO₂ depending on synthetic conditions and structural differences of amine sources*. Energy, 2011. **36**(2): p. 1243-1254.
238. Lv, K., et al., *(Bi, C and N) codoped TiO₂ nanoparticles*. Journal of Hazardous Materials, 2009. **161**(1): p. 396-401.
239. Sing, K.S.W., *The use of physisorption for the characterization of microporous carbons*. Carbon, 1989. **27**(1): p. 5-11.
240. Armandi, M., et al., *Study of hydrogen physisorption on nanoporous carbon materials of different origin*. International Journal of Hydrogen Energy, 2011. **36**(13): p. 7937-7943.
241. Sing, K.S.W., *Physisorption of gases by carbon blacks*. Carbon, 1994. **32**(7): p. 1311-1317.
242. Vijayan, B.K., et al., *Coupling Titania Nanotubes and Carbon Nanotubes To Create Photocatalytic Nanocomposites*. ACS Catalysis, 2011. **2**(2): p. 223-229.
243. Min, Y., et al., *Enhanced chemical interaction between TiO₂ and graphene oxide for photocatalytic decolorization of methylene blue*. Chemical Engineering Journal, 2012. **193-194**(0): p. 203-210.
244. Zhang, Y.-p., et al., *Preparation of graphene and TiO₂ layer by layer composite with highly photocatalytic efficiency*. Progress in Natural Science: Materials International, 2011. **21**(6): p. 467-471.
245. Wang, F. and K. Zhang, *Reduced graphene oxide-TiO₂ nanocomposite with high photocatalytic activity for the degradation of rhodamine B*. Journal of Molecular Catalysis A: Chemical, 2011. **345**(1-2): p. 101-107.
246. Zhang, K., et al., *Comparison of catalytic activities for photocatalytic and sonocatalytic degradation of methylene blue in presence of anatase TiO₂-CNT catalysts*. Ultrasonics Sonochemistry, 2011. **18**(3): p. 765-772.
247. Ma, L., et al., *In-situ fabrication of CNT/TiO₂ interpenetrating network film on nickel substrate by chemical vapour deposition and application in photoassisted water electrolysis*. Catalysis Communications, 2012. **21**(0): p. 27-31.
248. Morales, E.R., et al., *Physical properties of the CNT:TiO₂ thin films prepared by sol-gel dip coating*. Solar Energy, 2012. **86**(4): p. 1037-1044.
249. Chen, M.-l., F.-j. Zhang, and W.-c. Oh, *Synthesis, characterization, and photocatalytic analysis of CNT/TiO₂ composites derived from MWCNTs and titanium sources*. New Carbon Materials, 2009. **24**(2): p. 159-166.
250. Azadi, P., R. Farnood, and E. Meier, *Preparation of Multiwalled Carbon Nanotube-Supported Nickel Catalysts Using Incipient Wetness Method†*. The Journal of Physical Chemistry A, 2009. **114**(11): p. 3962-3968.
251. Mukul Kumar, y.A., *Chemical Vapor Deposition of Carbon Nanotubes: A Review on Growth Mechanism and Mass Production*. Journal of Nanoscience and Nanotechnology, 2010. **10**: p. 3739-3758.

252. Orendorz, A., et al., *Phase transformation and particle growth in nanocrystalline anatase TiO₂ films analyzed by X-ray diffraction and Raman spectroscopy*. Surface Science, 2007. **601**(18): p. 4390-4394.
253. Xu yong, S.M.A.A., *The Absolute Energy Positions of Conduction and Valence Bands of Selected Semiconducting Minerals*. American Mineralogist, 2000. **85**: p. 543-556.
254. Su, W.S., T.C. Leung, and C.T. Chan, *Work function of single-walled and multiwalled carbon nanotubes: First-principles study*. Physical Review B, 2007. **76**(23): p. 235413.
255. Velasco, M.J., et al., *Hydrolysis of Titanium Tetrabutoxide. Study by FT-IR Spectroscopy*. Spectroscopy Letters, 1999. **32**(2): p. 289-304.
256. Honda, H., K. Suzuki, and Y. Sugahara, *Control of Hydrolysis and Condensation Reactions of Titanium tert-butoxide by Chemical Modification with Catechol*. Journal of Sol-Gel Science and Technology, 2001. **22**(1-2): p. 133-138.
257. Liu, H., et al., *Self-Assembly of Large-Scale Micropatterns on Aligned Carbon Nanotube Films*. Angewandte Chemie International Edition, 2004. **43**(9): p. 1146-1149.
258. Lau, K.K.S., et al., *Superhydrophobic Carbon Nanotube Forests*. Nano Letters, 2003. **3**(12): p. 1701-1705.
259. Yan, X.-b., et al., *Fabrication of Three-Dimensional ZnO–Carbon Nanotube (CNT) Hybrids Using Self-Assembled CNT Micropatterns as Framework*. The Journal of Physical Chemistry C, 2007. **111**(46): p. 17254-17259.
260. Khranovskyy, V., et al., *Surface morphology effects on the light-controlled wettability of ZnO nanostructures*. Applied Surface Science, 2012. **258**(20): p. 8146-8152.
261. Liang, Q., et al., *Tailoring the wettability of nanocrystalline TiO₂ films*. Applied Surface Science, 2012. **258**(7): p. 2266-2269.