Plasma-controlled nanocrystallinity and phase composition of TiO₂: a smart way to enhance biomimetic response

Wei Zhou, Xiaoxia Zhong, Xiaochen Wu, Luqi Yuan, Qiwei Shu, Yuxing Xia, Kostya (Ken) Ostrikov

1Department of Physics, Shanghai Jiao Tong University, 200030 Shanghai, People’s Republic of China
2School of Physics, The University of Sydney, Sydney, New South Wales 2006, Australia

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Abstract: This contribution sheds light on the role of crystal size and phase composition in inducing biomimetic apatite growth on the surface of nanostructured titania films synthesized by reactive magnetron sputtering of Ti targets in Ar + O₂ plasmas. Unlike most existing techniques, this method enables one to deposit highly crystalline titania films with a wide range of phase composition and nanocrystal size, without any substrate heating or postannealing. Moreover, by using this dry plasma-based method one can avoid surface hydroxylation at the deposition stage, almost inevitable in wet chemical processes. Results of this work show that high phase purity and optimum crystal size appear to be the essential requirement for efficient apatite formation on magnetron plasma-fabricated bioactive titania coatings.

Key words: titanium oxide; reactive magnetron sputtering; bioactivity; nanocrystalline films; apatite formation

INTRODUCTION

Titanium and its alloys, such as Ti6Al4V, find numerous clinical applications as load-bearing orthopedic implants, owing to their excellent biocompatibility, mechanical properties, and chemical stability. Unfortunately, titanium-based materials are bioinert, and generally a connective tissue capsule would be formed around bioinert materials. To improve the surface reactivity (bioactivity), the titanium-based implant are coated with a thin film of a bioactive material such as hydroxyapatite (HA), bioglass, and silica (SiO₂). The reactivity of the titanium can also be enhanced by titania (TiO₂) coatings, because of their capability to induce calcium phosphate formation in vitro and in vivo. In addition, it has been reported that the adhesion between titania coatings and titanium substrate are sufficient for their use as implant coatings. The formation of biologically active layer on implant surface is considered crucial for their integration with bone, and materials having the capability of inducing calcium phosphate layer formation on their surfaces are often referred to as bioactive materials. Every specific bioactive material has different factors that induce and control their bioactive response. Generally, such factors include reactivity, morphology, roughness of the surface, crystal size, phase composition, stoichiometry, and some others.

This article contributes to answering the question which has puzzled the minds of researchers in the last couple of decades, namely, what is the role of crystal size and phase composition in sustaining the outstanding biomimetic response of titania coatings? It is generally believed that the remarkable biomimetic response of TiO₂ is attributed to the existence of surface hydroxyl (Ti-OH) groups and the induced negative charges on the TiO₂ surface, which, in turn, draw calcium- and phosphorus-based cations from the simulated body fluid (SBF) to the implant surface.

However, this growth model does not explain frequent observations of different biomimetic responses of amorphous, rutile- and anatase-rich titania films derived from conventional wet methods, such as sol–gel and other chemical methods. These techniques almost inevitably lead to pronounced surface hydroxylation during the film synthesis stage. In these cases,
the TiO$_2$ surface is grafted with hydroxyl groups even before immersion in the SBF, which make it extremely difficult to find out the effects of crystal size and phase composition on the TiO$_2$ biomimetic response in the SBF. Moreover, postannealing of hydroxyl-terminated titania films often results in a different abstraction of hydroxyl groups from the anatase and rutile phases, leaving some of the sites on the TiO$_2$ surface-activated and some passivated. Indeed, higher postannealing temperatures are needed to convert amorphous TiO$_2$ films (synthesized by using wet chemical methods) into the rutile phase rather than into the anatase phase. Thus, at lower temperatures, when the anatase phase is enriched, more hydroxyl groups remain on the surface than at higher temperatures when the rutile phase is formed. The excessive Ti-OH groups might be the main reason for frequently reported stronger biomimetic responses of the anatase-rich titania coatings in the SBF in vitro tests. Therefore, the question of whether the anatase phase is more bioactive than the rutile phase in the SBF environment still remains essentially open.

It becomes evident that the reliable and unambiguous information about the effects of crystal size and phase composition on the biomimetic responses can be obtained if the as-grown films with different crystal size or phase composition are free of surface hydroxyl radicals. In this article, we propose to use a dry magnetron plasma-based method to fabricate nanocrystalline and hydroxyl-free titania films with the controlled anatase and rutile phase composition and nanocrystal size. This technique has recently been used for fabricating biocompatible HA and calcium phosphate coatings, semiconducting nanostructured films, and low-dimensional quantum confinement structures. We are not aware of any reports related to the in vitro bioactivity of TiO$_2$ films deposited by reactive magnetron sputtering. Here, we report on the reactive DC magnetron sputtering deposition of titania films in Ar+O$_2$ plasmas on unheated substrates. It is shown that the degree of crystal size and phase composition can be controlled by the process parameters and are unambiguously related to the biomimetic response in the SBF test environment.

**MATERIALS AND METHODS**

**Deposition and characterization of TiO$_2$ films**

Polished Si(111) wafers have been used as substrates for depositing the TiO$_2$ films. Prior to deposition, the substrates were ultrasonically cleaned in acetone, followed by ethanol, and finally, deionized water for 15 min in each solution and dried thereafter. The TiO$_2$ films were grown by reactive DC magnetron sputtering technique, without any external substrate heating. Substrates were mounted on a water-cooled substrate stage that could continuously revolve without disrupting the vacuum in the system. A mask plate with a 50-mm diameter hole was placed between the substrate and the target electrode and served as a shutter, which made it possible to condition the target surface before film deposition. Continuous rotation of samples over the sputtering target enabled us to grow several samples under identical vacuum conditions. The distance between the sputtering target and deposition substrates was 90 mm. The purity of the titanium target (FHR Anlagenbau GmbH) with 60-mm diameter and 3-mm thickness mounted on the magnetron cathode was 99.9%. Local magnetic field strength measured above the target was in the range 2500–2800 G. A pumping system was used to achieve a base pressure below 1.0 × 10$^{-4}$ Pa. High-purity argon (99.999%) and oxygen (99.5%) were used as sputtering and reactive gases, respectively. The gas handling system included MKS mass flow controllers that were used to control the flow rates and partial pressures of argon and oxygen in the reactor chamber.

Our choice of deposition conditions was motivated by the aim to synthesize nanocrystalline TiO$_2$ films with the required crystal size and rutile/anatase phase composition, and ultimately, to relate the varied film attributes to their biomimetic response. In a sense, we explored a number of possible "bioactivity turning knobs" of the plasma-assisted process. Likewise, our choice of sputtering and reactive gases effectively excludes any hydrogen source, and thus prevents the formation of hydroxyl groups on specimen surfaces, and therefore is favorable for a hydroxyl-free deposition environment. In this set of experiments, the O$_2$ and Ar flow rates were maintained at 5 and 30 sccm, respectively. Furthermore, the titania films were synthesized under five different sets of process conditions.

Sample T0V was grown at a total pressure of 1.2 Pa, with a deposition power of 40 W (power supplied to the DC magnetron) and without any external substrate biasing. Meanwhile, sample T80W was deposited at the same working pressure, but with doubled deposition power (P$_D$ = 80 W) and a negative substrate bias of −100 V. The other three samples, T03P, T12P, and T36P, were fabricated at the same deposition power as T0V (P$_D$ = 40 W), the same DC substrate bias (V$_s$ = −100V) as T80W, and different total pressures of 0.3, 1.2, and 3.6 Pa, respectively. Table I details the process conditions specific to each of the samples. Film thickness measured by the spectroscopic ellipsometry was in the range of 63–79 nm, as can be seen in Table I.

For crystalline structure analysis, a thin film X-ray diffractometer (Rigaku TF-XRD, D/max 2550VB/PC) was used with Cu K$_\alpha$ radiation, and it was operated at 40 kV, 300 mA, with a grazing incidence angle of 0.9° and with a scanning speed of 8°/min at 20 steps of 0.020°. The average sizes of anatase and rutile crystallites were estimated according to Scherrer’s equation (6) that relates crystal sizes to positions and full widths at half-maximum of diffraction peaks characteristic to the anatase and rutile phases of TiO$_2$.

The morphology of the outermost surface layer was studied by means of atomic force microscopy in a tapping mode (AFM, Nanoscope® III). The root-mean-square (RMS) roughness of the surface was calculated from AFM scans over substrate areas of 2 × 2 μm$^2$, and the RMS roughness was calculated three times, at different spots for each sample.

The chemical bonding states in the films of our interest were evaluated by using Fourier transform infrared spectroscopy

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**Table I**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Process Conditions</th>
</tr>
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<tbody>
<tr>
<td>T0V</td>
<td>Total pressure: 1.2 Pa, deposition power: 40 W, no external substrate biasing</td>
</tr>
<tr>
<td>T80W</td>
<td>Total pressure: 1.2 Pa, deposition power: 80 W, negative substrate bias: −100 V</td>
</tr>
<tr>
<td>T03P</td>
<td>Total pressure: 0.3 Pa, deposition power: 40 W, negative substrate bias: −100 V</td>
</tr>
<tr>
<td>T12P</td>
<td>Total pressure: 1.2 Pa, deposition power: 40 W, negative substrate bias: −100 V</td>
</tr>
<tr>
<td>T36P</td>
<td>Total pressure: 3.6 Pa, deposition power: 40 W, negative substrate bias: −100 V</td>
</tr>
</tbody>
</table>
FTIR, EQUINOX-55, Bruker) with a resolution of 0.8 cm\(^{-1}\), within the spectral range of 400–4000 cm\(^{-1}\). In particular, the FTIR technique enabled us to monitor the presence of the hydroxyl (OH) groups on the surface.

Finally, the aqueous wettability of the deposited TiO\(_2\) film was analyzed by using standard routines for measuring the equilibrium water contact angle. The wettability tests were repeated three times for each specimen.

**In vitro biomimetic response experiments**

The SBF was prepared by dissolving reagent-grade chemicals NaCl, NaHCO\(_3\), KCl, K\(_2\)HPO\(_4\)·3H\(_2\)O, MgCl·6H\(_2\)O, CaCl\(_2\), and Na\(_2\)SO\(_4\) in distilled water, and then buffered at pH 7.40 with (CH\(_2\)OH)\(_3\)CNH\(_2\) and HCl at 36.5°C. The ion concentrations of SBF are nearly equal to those of human blood plasma at 36.5°C. The as-deposited TiO\(_2\) films were soaked in SBF solutions by using a surface area to solution volume ratio of 0.1 cm\(^{-1}\). After soaking for 7 days, the specimens were taken out from the SBF, washed with distilled water, and dried in air.

Scanning electron microscopy (SEM, Sirion 200, Philips) was used to examine the surface morphology of the TiO\(_2\) surface after soaking in SBF. To avoid undesired charge buildup on apatite surfaces, samples were partially coated with gold. The details of the X-ray diffractometry and the FTIR analysis are essentially the same as in Materials and Methods.

**RESULTS**

**Deposition of TiO\(_2\) films by reactive magnetron sputtering**

In this study, we have used five TiO\(_2\) samples deposited under different process conditions summarized in Table I. Samples T03P, T12P, and T36P were synthesized under the same deposition (sputtering) power \(P_D = 40\) W and negative DC bias on the substrate \(V_s = -100\) V and different working pressures (\(p_0 = 0.3, 1.2,\) and 3.6 Pa, respectively). Sample T0V was fabricated under the same pressure and sputtering power as T12P, but without any external biasing of the substrate. Finally, specimen T80W was deposited under the same working pressure and substrate bias as T12P and a higher sputtering power of 80 W. This choice of process parameters enabled us to synthesize titania films with different crystal sizes and phase compositions. Table I also summarizes the average film thickness and sizes of An(101) and Ru(110) crystals, in each specimen.

Figure 1 shows X-ray diffraction (XRD) patterns of films synthesized under the process conditions of Table I. Film T03P (deposited at \(p_0 = 0.3\) Pa, \(P_D = 40\) W, \(V_s = -100\) V) features a wide diffraction peak of Ru(110), originating from very small rutile nanocrystals with an estimated grain size of \(\sim 3.2\) nm. Since XRD analysis of sample T03P does not reveal any other diffraction peaks, it is reasonable to assume that this film is composed of either a pure rutile phase or a...
mixture of crystalline rutile and amorphous titanium oxide.

When the total pressure increases from 0.3 to 1.2 Pa, no diffraction peaks have been detected (Fig. 1) in film T12P (deposited at $p_0 = 1.2$ Pa, $P_D = 40$ W, $V_s = -100$ V). Thus, the film T12P is either purely amorphous or contains ultrasmall nanocrystallites undetectable by XRD.

However, diffraction patterns of the film T36P deposited at a higher working pressure of 3.6 Pa and the same $V_s$ and $P_D$ show pronounced peaks corresponding to An(101), An(103), An(004), An(200), An(211), and An(204) crystalline planes (Fig. 1). In this case, TiO$_2$ has an anatase structure with an estimated nanocrystal size of 19.1 nm (Table I). Furthermore, the intensity of the An(101) peak is the highest, which is indicative of preferential crystal growth along the (101) crystallographic direction.

An increase of magnetron sputtering power results in dramatic changes in film composition and structure. Indeed, sample T80W, deposited at a higher ($P_D = 80$ W) magnetron sputtering power and with all other parameters same as T12P, shows a mixed-phase crystalline structure with estimated anatase and rutile nanocrystal sizes of 18.6 and 16.3 nm, respectively. Amongst the variety of diffraction peaks seen in Figure 1, the An(101) and Ru(110) peaks are the strongest. Thus, the most efficient growth of anatase crystals proceeds along the (101) direction, similar to sample T36P. On the other hand, the preferential growth direction of rutile crystals in specimen T80W is quite different from their growth under T03P conditions.

The sole An(101) peak also persists in the XRD spectrum (the upper curve on the top panel in Fig. 1) from film T0V synthesized without any external DC biasing of the substrate. However, this peak is much weaker and wider, and suggests the presence of anatase nanocrystals with a size of ~6.8 nm.

Figure 2 displays the FTIR transmission spectra of the TiO$_2$ films deposited on unheated silicon substrates under the deposition conditions of Table I. The strong absorption peak at ~610 cm$^{-1}$ and two weak absorption peaks around 740 and 815 cm$^{-1}$ are present in all samples, including the pure Si(111) test sample, and are thus attributed to infrared absorption by the substrate. On the other hand, strong absorption of Ti—O vibrations in the 400–600 cm$^{-1}$ spectral range suggests the presence of Ti—O bonds in the deposited material.$^{25}$ In this experiment, specimens T36P and T80W with larger crystal sizes exhibit a much stronger infrared absorption in this spectral region compared to films T0V and T03P featuring smaller crystal sizes. Moreover, the amorphous TiO$_2$ film of sample T12P shows almost no infrared absorption in the 400–600 cm$^{-1}$ range, very similar to the silicon test sample. One can thus conclude that TiO$_2$ films with larger nanocrystal sizes feature stronger bonding between titanium and oxygen atoms, which is reflected by intense infrared absorption peaks in the relevant infrared spectral range.

More importantly, the FTIR spectra in Figure 2 do not show any infrared absorption lines characteristic to vibrations of hydroxyl groups. Indeed, typical FTIR spectra of titania films synthesized by wet chemical methods feature absorption bands around 3400 cm$^{-1}$ and 3700–3800 cm$^{-1}$, which are completely absent in our experiments. Therefore, the TiO$_2$ films synthesized in this work by plasma-assisted reactive magnetron sputtering without any external substrate heating and postannealing are free of surface hydroxyl (Ti-OH) groups.

Figure 3 shows three-dimensional surface morphology of the TiO$_2$ films imaged by the AFM in a tapping mode over the surface area 0.5 x 0.5 mm$^2$. For the convenience of the reader, process parameters are shown separately for each of the samples. The RMS surface roughness derived from AFM analysis is presented in the upper panel in Figure 1. From Figures 3 and 4, one can conclude that the surface roughness of samples T36P and T80W is the largest, followed by T0V, T03P, and amorphous T12P. Comparison of the results in Table I and Figures 3 and 4 shows a remarkable correlation between the surface roughness and the estimated size of the nanocrystals. Specifically, films T36P and T80W with the largest nanocrystal size are the roughest, whereas the films T0V and T03P with smaller crystal sizes are smoother. Meanwhile, the amorphous
T12P film appears to be the smoothest. Generally speaking, a larger crystalline size corresponds to a rougher surface morphology. However, for all samples, the surface roughness remains smaller than the estimated nanocrystal size, indicating the presence of amorphous overcoats covering the nanocrystalline matter. The surface roughness is intimately related to the wettability of the coating, which is characterized by the water contact angle. Figure 4 shows the equilibrium water contact angles for the TiO₂ films deposited at different process conditions. Interestingly, the measured values of the contact angle for all of the specimens fall within the range of 87–95°, which is consistent with the reports of other authors.²⁶ Reasonably high values of the contact angle suggest low surface energy of the films synthesized by the plasma-assisted magnetron sputtering technique.

**In vitro apatite formation**

In this section, we consider the bioactivity properties of TiO₂ films evidenced by the apatite crystal ingrowth during specimen immersion in the SBF. Figure 5 shows the TF-XRD patterns of the samples after soaking in the SBF for 7 days. The most prominent (211) diffraction peak at 2θ = 32°, attributed to the apatite crystalline structure, has been observed in the XRD spectra of the anatase TiO₂ films of samples T36P and T0V. Moreover, the intensity of the apatite diffraction peak is higher for the T36P film with the largest anatase nanocrystal sizes. No other calcium phosphate phases were observed in the XRD spectra in Figure 5.

Figure 6 shows the FTIR transmission spectra of the TiO₂ films after soaking in the SBF for 7 days. After exclusion of the absorption peaks around 610, 740, and 815 cm⁻¹ characteristic to the Si(111) substrate, we have found a number of infrared absorption bands that evidence the presence of new materials that contain the PO₄ groups and grow on the surfaces of the TiO₂ films in the SBF environment. Among numerous infrared absorption peaks denoted in Figure 6, the absorption peak ~464 cm⁻¹ reflects the v₂ P–O twofold degenerate deformational mode vibrations of PO₄ groups in phosphate; the band at ~564 cm⁻¹ represents the v₄ P–O threefold degenerate deformational mode vibrations; the peak around 962 cm⁻¹ is due to the v₁ P–O symmetric stretching mode vibrations; and the bands in the 960–1200 cm⁻¹ range result from the v₄ P–O threefold degenerate stretching mode vibrations.²⁷

Thus, Figure 6 confirms that apart from the anatase TiO₂ films of samples T36P and T0V, the rutile TiO₂ film of sample T03P and the anatase/rutile mixed TiO₂ film of sample T80W also induce nucleation (in the SBF) of materials containing PO₄ groups on the surface. More importantly, no changes have been found in the FTIR spectra of the amorphous TiO₂ film of sample T12P immersed in SBF for 7 days.

Figure 7 shows SEM micrographs of the surfaces of TiO₂ films deposited at different deposition conditions and soaked in SBF for 7 days. A notable apatite precip-
Pitiation has been observed on the surfaces of the three samples; two of them have an anatase structure \([\text{T0V, Fig. 7(a) and T36P, Fig. 7(c)}]\) and the third one has a rutile structure \([\text{T03P, Fig. 7(e)}]\). From Figure 7(b), related to sample T80W with the presence of mixed anatase/rutile phases, one can notice a low degree of surface coverage by irregularly shaped particles. Presumably, such particles contain the PO\(_4\) groups, as evidenced by the FTIR analysis detailed earlier. The most striking observation is the complete absence of any precipitation from the SBF onto the surface of the amorphous film T12P \([\text{Fig. 7(d)}]\). In this case, only loosely attached contaminant particles of irregular shapes have been observed. On the rutile surface of sample T03P with a small crystal size of 3.2 nm, apatite particles with an average size of \(\sim 1\) \(\mu\)m partially cover the surface (with the average surface coverage not exceeding 0.2) as shown in Figure 7(e).

As can be seen in Figure 7(c), the anatase TiO\(_2\) film of sample T36P with a large crystal size of 19.1 nm induced the strongest apatite formation. The high-resolution SEM image in Figure 8(b) suggests that the thickness of the apatite layer formed on the surface of the anatase film T36P exceeds 1 \(\mu\)m. Moreover, the porous microstructure of the apatite layer in Figure 8(b) is very similar to that reported elsewhere.\(^{8-14,19,20}\) A continuous layer of porous apatite was also formed on the surface of the anatase film of sample T0V with a smaller crystal size of 6.8 nm. However, this layer is thinner and less dense as can be seen in Figure 8(a). To conclude this section, it is worth noting that SEM (Figs. 7 and 8) reveals similar trends, as suggested by the XRD and FTIR results shown in Figures 5 and 6.

**DISCUSSION**

**Growth kinetics of TiO\(_2\) films**

We now discuss the growth kinetics of titania films synthesized by the plasma-assisted reactive magnetron sputtering process. To elucidate the elementary processes that occur in the ionized gas phase and on the deposition surfaces, we adopt the generic “plasma-building unit” approach that is applicable to a wide...
variety of plasma-assisted material synthesis and processing applications. The overwhelming complexity of possible sequences of multiple elementary reaction steps makes the analysis particularly difficult. However, by sorting out the possible elementary building blocks and their dynamics in the plasma sheath and on the solid surfaces, one can develop reasonable scenarios for virtually any process of synthesis of various thin films and nanoassemblies. Drawing from the available literature on plasma-assisted synthesis of TiO₂ clusters, films, and nanoparticles, we consider the following range of building and other functional units (species) that originate in the ionized gas phase: Ti atoms; positive ions Ti⁺, Ti²⁺, and Ti³⁺; oxygen atoms, molecules, and ions (including anions); nonreactive argon neutrals and energetic argon ions; amorphous titania clusters with the sizes less than 2 nm and crystalline clusters larger than 3 nm. Earlier we have noted that the amorphous-to-crystalline transition in TiO₂ nanoclusters takes place when the cluster size exceeds ~2 nm.

Each of the species listed earlier serves a specific purpose. Specifically, Ti, Ti⁺, Ti²⁺, and Ti³⁺ are the titanium source species; oxygen species serve as oxidizing reagents needed for the synthesis of titania; TiO₂ nanoclusters are important building blocks that directly incorporate into growing films; energetic argon atoms activate and heat the surface. Likewise, low-energy (room temperature) neutral argon atoms are nonreactive, and whilst they do not participate directly in the film growth process, they strongly affect (via elementary ion–neutral and neutral–neutral collision processes) the transport of titanium species from the sputtering target to the deposition surface. Following the charged cluster model, we assume that the nanocluster charge is positive in the specified size range. The presence of charged clusters in the few nanometers size range has been invoked by comparing the deposition conditions in our experiments, with available results reporting the size dependence of the plasma-grown TiO₂ nanoclusters on the process parameters in a fairly similar plasma-assisted reactive magnetron sputtering process. The assumption of positive charge is also consistent with other reports on plasma-generated positively charged clusters and nanocrystallites of other materials in this size range. Another important point frequently sidestepped in plasma-assisted materials synthesis and processing works is the fact that the plasma bulk is separated from the solid surface by the nonneutral plasma sheath area, which is collisionless under the low-pressure conditions of our experiments.

The TiO₂ growth kinetics are sketched in Figure 9, which illustrates the effect of changing process conditions on the structure and phase composition of the films of our interest here. We recall that the T03P sample has been synthesized under a low pressure (0.3 Pa), “normal” (40 W) deposition power and “normal” (−100 V) bias. In this case, sketched in the top left drawing in Figure 9, energetic argon ions heat and activate the surface. The background pressure is rather low for the formation of titania molecules and clustering in the gas phase. Since the pressure of bombarding argon ions is low, the influx of titanium atoms and ions onto the deposition surface is also low; upon adsorption they become adatoms and diffuse, together with oxygen atoms, about the silicon surface, to nucleate and form nanosized islands on the surface. In this case, the growth islands develop into small rutile nanocrystallites. Under the low-pressure condition T03P, formation of rutile appears to be energetically favorable. It is instructive to mention that rutile is the most stable form of TiO₂, and its formation is preferred under equilibrium conditions. Moreover, the critical nucleus size needed for crystallization is smaller for the rutile phase than for the anatase phase. In this case, the supply of titanium-bearing building blocks is too low to create the critical nucleates suitable for crystallization of the anatase phase. This conclusion is consistent with other reports on preferential synthesis of rutile under very similar pressures, for example, 0.27 Pa.

Figure 6. FTIR transmission spectra of the TiO₂ films after immersion in SBF for 7 days.
Upon a fourfold increase of the working pressure, the density of argon and oxygen neutral species increases accordingly in depositing sample T12P. Since the heat generated in the plasma process could be efficiently transferred out of the deposition chamber by the water-cooling system, the increase of the process pressure does not lead to any significant changes in the temperature of the gas (which thus remains at room temperature) in thermodynamic equilibrium. According to Pashen’s curves, in this pressure range, the density of the energetic argon ions that are mainly responsible for the sputtering of the titanium target also increases. Thus, sputtering of the titanium target becomes more efficient giving rise to a larger number of titanium-containing building blocks supplied to the deposition site. This effect is somewhat hindered by the collisions of Ti atoms and ions with the argon gas maintained at a higher (than in the case T03P) pressure. However, since we have assumed that the argon gas temperature does not significantly change at higher pressures, the titanium species arrive at the silicon surface with the same kinetic energy as in case T03P, as a result of rapid thermalization in the ambient room-temperature gas.

Therefore, the supply of titanium-bearing building units to the growth surface in the case T12P is likely to be excessive, which results in purely amorphous films; in this case, the rates of the surface diffusion are insufficient to redistribute the deposited adatoms over the surface; excessive piling up of the building material results in a significant disorder in the films. It is quite possible that the pressure (1.2 Pa) is too low to induce any noticeable growth of larger crystalline clusters (≥3 nm) in the gas phase, and there are mainly small (<2 nm) positively charged amorphous TiO₂ clusters in the gas phase. Such clusters contribute to the higher content of the amorphous phase. Moreover, all crystalline clusters with cohesive energies less than 100 eV most likely break into smaller fragments

Figure 7. SEM micrographs of the surfaces of TiO₂ films after immersion in SBF for 7 days.

Figure 8. SEM images of the surface morphology of the apatite layers deposited on anatase TiO₂ films of sample T0V and sample T36P after immersion in SBF for 7 days.
upon deposition on the biased Si(111) surface. This disintegration of crystalline nanoclusters further contributes to the buildup of the amorphous phase.

When the pressure is increased to 3.6 Pa (sample T36P, top right sketch in Fig. 9), the supply of titanium-bearing building material further increases and becomes sufficient to generate larger (supercritical) nucleates, both in the gas phase and on the surface. Crystalline clusters with cohesive energies exceeding 100 eV are likely to form in the gas phase; they do not break, upon landing on biased silicon surfaces. In this case, the film can contain a large amount of anatase nanoclusters embedded in the amorphous matrix. It is remarkable that other authors also reported purely anatase films in the pressure range exceeding 2.7 Pa.33

Specimen T0V was synthesized under the same magnetron sputtering power and gas pressure as the purely amorphous sample T12P, but without any external biasing of the substrate (sketch in the middle of the top row in Fig. 9). In this case, the argon ion bombardment is significantly reduced, and the surface temperature becomes lower than in cases T03P and T12P. This condition becomes even less favorable for the rutile phase formation (as compared to conditions T12P and T03P) as higher surface temperatures are usually required for the synthesis of rutile crystalline films. Since the substrate is unbiased, the gas-phase grown charged clusters do not disintegrate upon landing, and the film contains a notable amount of small nanocrystallites, which is quite similar to T36P. Under such conditions, the surface temperature becomes lower than necessary to form rutile crystals via adatom migration about the surface (as was the case for T03P). This lower surface temperature reduces the critical nucleate size for the anatase phase crystallization, and it becomes possible to synthesize smaller anatase crystals (as can be seen in case T0V). However, it is already not possible to grow the rutile crystals, as the temperature is lower and the amount of supplied Ti atoms and ions is approximately the same as in case T12P.

In the T80W case, the gas pressure and substrate bias are the same as in T12P, but the magnetron sputtering power is doubled from 40 to 80 W. In this case, depicted in the bottom left drawing in Figure 9, the plasma density substantially increases, as do the electron-impact ionization rates of argon, oxygen, and titanium species. Stronger fluxes of Ar⁺ ions result in the highest (among the five specimens of our interest here) surface temperatures. This makes it possible to significantly enhance the rates of surface diffusion, and thus avoid piling-up of deposited building material, as was the case under process conditions T12P. This enhances the nucleation of islands on the surface (Volmer–Weber growth mode in lattice mismatched systems). If the island growth is unobstructed by piling up of undesired amorphous deposits, the resulting island size distribution is usually Gaussian-like.35 Therefore, in this case, there should be sufficient number of nucleates of a suitably small size to act as growth seeds of rutile crystallites. Thus, rutile nanocrystals are also present in the film, similar to case T03P. On the other hand, small charged nanoclusters grown in the gas phase also break upon deposition, as happens under the T12P process conditions. However, in this case, the surface temperature is already

Figure 9. Growth kinetics of TiO₂ films in the plasma-assisted reactive magnetron sputtering deposition.
high enough and there is a sufficient amount of supercritical nucleates for the efficient adatom diffusion-driven crystallization on the surface.

It is important to note that an excessive increase of the bias should increase the likelihood of energetic-ion induced disorder in the TiO₂ films. Indeed, if the energy of the impinging argon ions exceeds the lattice cohesive energy, the crystalline structure can be damaged and the films can become amorphous. This situation is depicted in the bottom right drawing in Figure 9. However, if the rates of crystallization are larger than those of the crystalline structure damage, crystalline structures are still possible. Therefore, there is a tradeoff between higher surface temperatures and crystalline structure destruction caused by energetic argon ions. Furthermore, heating of the surface to 915°C (by either applying a larger bias or an external substrate heating) will result in the phase transformation of any existing anatase crystals into the more stable rutile form. Finally, we note that our observations of structural and phase transformations in titania films are consistent with the earlier report of Zeman et al.36

**Effect of crystal size and phase composition on biomimetic response of TiO₂ films**

We now comment on the roles of crystal size and phase purity/composition in inducing bioactivity of the titania films synthesized by the plasma-assisted reactive magnetron sputtering deposition technique. It is commonly believed that the existence of surface hydroxyl (Ti-OH) groups is crucial for apatite precipitation on the surfaces of titanium oxide films in the SBF. After immersion in the SBF with neutral pH (pH 7), acidic reaction instead of basic reaction dominates on the surface of TiO₂ film, since the acidic hydroxides are deprotonated.

\[
\text{Ti-OH (acidic hydroxide) + H}_2\text{O} \leftrightarrow \text{[Ti-O]}^- + \text{H}_3\text{O}^+
\]

which results in an induced negative charge on the TiO₂ surface. The negatively charged Ti-OH⁻ groups serve as precipitation sites for positive calcium and calcium oxide cations to form calcium titanate or calcium titanate oxide. As a result of positive charge buildup, the surface gradually becomes positively charged. In turn, such positively charged surface attracts negatively charged phosphate ions that combine with calcium-bearing species to form amorphous calcium phosphate with a low [Ca]/[P] ratio. It is also commonly presumed that the amorphous calcium phosphate formed as a result of surface hydroxyl-induced precipitation, spontaneously transforms into bone-like apatite (which is essentially crystalline) by continuously consuming calcium and phosphate ions in the SBF. Therefore, the existence of Ti-OH groups on the surface is widely considered to be the most essential prerequisite for successful formation of all kinds of bioactive TiO₂ films.6

For this reason, substantial research efforts were focused on intentional grafting of Ti-OH groups onto titania surfaces, for example, by sol–gel chemical methods, heat treatment in water vapor, or hydrogen plasma treatment. However, the existing nucleation models merely based on grafted Ti-OH groups do not include any charge neutral pathways and also cannot explain why amorphous TiO₂ films produced by existing wet methods do not show any significant biomimetic response (there is generally a large amount of Ti-OH groups on the surface of TiO₂ films produced by wet methods). None of these models shed any light on how specifically and how fast the disordered amorphous precipitates transform into crystalline apatite to stand a chance of mimicking natural bone apatites. From a clinical application point of view, crystallization should be induced instantaneously, without any intermediate resorption frequently observed in in vivo SBF tests, involving HA bioceramics.22

To single out the effect of crystal size and phase composition on the bioactivity of TiO₂ films, during the synthesis stage, we intentionally avoided the formation of Ti-OH surface groups, almost unavoidable in wet chemical methods. Nevertheless, several samples exhibited a notable bioactivity in the SBF, as is clearly seen in Figures 5, 6, and 7(a,c,e).

We emphasize that without the interference from Ti-OH surface groups grafted during the film synthesis stage one can unambiguously investigate the effect of crystal size and phase composition on the bioactivity of the deposited TiO₂ films. In this study, we managed to synthesize five remarkably different samples. Two of them are purely anatase and contain nanocrystals of different sizes (T36P and T0V). Sample T03P is a phase-pure rutile, whereas sample T80W is a mixture of rutile and anatase phases. Finally, sample T12P is amorphous. We recall that the best biomimetic response was generated by the purely anatase samples T36P and T0V [Fig. 7(a,c)]. Interestingly, the TiO₂ surface coverage by the apatite material is higher in the T0V case [Fig. 7(a)], when the estimated anatase nanocrystal size is smaller (~6.8 nm). On the other hand, the film thickness and surface morphology feature sizes are larger under process conditions T36P. This observation can be explained in two aspects.

First, with the decrease of crystal size, there are more crystal boundaries, defects, and monoatomic terraces that can serve as sites for the apatite nucleation and subsequent crystallization in the SBF. Lattices of smaller crystals are strained and are generally more energetically favorable for new building units to insert and recrystallize in the existing crystal structure. However, it is more difficult to insert into larger crystals with established and strain-relaxed lattices. This can be the reason why (nucleation-site-free) single-crystalline anatase exhibits no bioactivity.
Second, the lattice match (both lattice parameters and orientation) is an important factor that controls epitaxial growth and recrystallization of apatite crystals from initial nucleation sites on TiO₂ surfaces. When the crystallinity is low and the nanocrystal size is small, the mutual disorder of the apatite and titania lattices increases, mostly because of the higher structural disorder on the surface. This disorder is highest in amorphous titania, featuring chaotically oriented lattice nanofragments. Moreover, the surface strain becomes so irregular, so that the Volmer–Weber mechanism applicable to the crystal growth in lattice-mismatched crystalline systems can no longer sustain the apatite crystallization. This explains, in part, why amorphous TiO₂ layers always fail to induce any apatite formation during immersion in the SBF, which can be seen in Figure 7(d) and was reported elsewhere.

Therefore, there is a tradeoff between crystal size, surface defects, and structural disorder of anatase TiO₂ films to induce the best in vitro biomimetic response. Our results also suggest that the phase purity of the titania films is an important element in sustaining the required apatite precipitation. Indeed, the phase-mixed TiO₂ film of specimen T80W did not induce any significant apatite ingrowth. This observation can be interpreted, in part, in terms of an increased structural disorder on the surfaces of phase-mixed systems. However, it still remains unclear as to why the apatite does not follow the polynanocrystalline growth scenario peculiar to the mixed-phase anatase/rutile film of sample T80W.

Although only a little apatite formation was observed after the 7-day soaking of sample T03P in SBF, this observation does not necessarily indicate the conclusion that the biomimetic response of rutile phase is worse than that of the anatase phase, as several existing reports suggest. Perhaps, it is the very small nanocrystal size (~3.8 nm) of film T03P that led to such weak biomimetic response. From our point of view, only when the crystal size of the studied TiO₂ films is similar and the interference of initial Ti-OH groups on their surface is excluded, it is possible for researchers to compare biomimetic responses between anatase phase and rutile phase not only in vitro but also in vivo.

Finally, our experiments did not reveal any apparent relationship between in vitro bioactivity in the SBF on one hand and surface roughness and water contact angle (Fig. 4) on the other. It is worthwhile to mention that surface roughness as well as surface wettability have been found to have a significant influence on in vitro bioactivity of the TiO₂ films with amorphous structure in our previous work. The observation in this work is reasonable if one notes that the wettability of all the samples in this experiment is approximately the same and their in vitro bioactivity is more likely to be controlled by other important factors such as nanocrystal size and phase purity.

CONCLUSIONS

In this study, by using an advanced plasma-assisted DC magnetron sputtering deposition on unheated substrates, TiO₂ films, free of surface hydroxyl groups, could be fabricated with different nanocrystal size and anatase/rutile phase composition. By using the plasma-building unit approach, one can explain the growth kinetics of the films with the required crystal size and phase purity, and select appropriate sets of process parameters. For example, by increasing the working gas pressure from 0.3 to 3.6 Pa, it is possible to switch the process output from the pure-phase ultrananocrystalline rutile to pure-phase nanocrystalline anatase.

By analyzing the biomimetic responses of all of the specimens in the SBF environment, we have presented evidence that phase-pure anatase films feature the best biomimetic response, which is evidenced by the pronounced apatite precipitation, nucleation, and crystallization in the SBF, and also depends on the TiO₂ nanocrystal size. Bioactive responses of mixed-phase anatase/rutile and ultrananocrystalline rutile appear to be weaker. Furthermore, since neither amorphous nor single-crystalline anatase can induce the apatite formation in the SBF, we conclude that high phase purity and structural order, and also optimum nanocrystal size and surface density of nucleation sites appear to be the essential requirements for efficient apatite crystallization on magnetron plasma-fabricated bioactive titania coatings.

In this experiment, all the TiO₂ films produced by reactive magnetron sputtering have almost no initial hydroxyl groups on their surfaces, but several of them still exhibit intense biomimetic response in SBF. Therefore, we can speculate that the existence of initial hydroxyl groups on the surface may not be the main decisive factor in inducing the positive bioactive response of bio compatible titania films, as is commonly believed.

Finally, we emphasize that dry plasma-based methods have several unique advantages over most of conventional wet chemical methods: (1) Without interference from initial surface hydroxyl groups that are almost unavoidable in most of the wet processes in particular, researchers can study more easily the effect of crystal structure (such as crystal size and phase composition) of the TiO₂ films on their bioactivity not only in vitro but also in vivo; (2) In the plasma process, researchers can deposit TiO₂ films with a wide range of crystal size and phase composition by control of a series of process parameters, such as deposition power, total pressure, oxygen partial pressure, substrate bias voltage, distance between substrate and titanium target, and so forth. (3) Since the deposition can be operated at room temperature without substrate heating or postannealing through the use of plasma-based methods, it becomes possible to coat...
bioactive TiO\textsubscript{2} films on many kinds of temperature-sensitive materials. This can boost the interest of the R&D community and biomedical industry toward the plasma-assisted fabrication of biomaterials, in particular, temperature-sensitive and cost-efficient materials such as polymers and plastics.

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References