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Stability and prospect of UV/H₂O₂ activated titania films for biomedical use

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Abstract

Biomedical implants and devices that penetrate soft tissue are highly susceptible to infection, but also accessible for UV induced decontamination through photocatalysis if coated with suitable surfaces. As an on-demand antibacterial strategy, photocatalytic surfaces should be able to maintain their antibacterial properties over repeated activation. This study evaluates the surface properties and photocatalytic performance of titania films obtained by H_2O_2 -oxidation and heat treatment of Ti and Ti-6Al-4V substrates, as well as the prospect of assisting photocatalytic reactions with H_2O_2 for improved efficiency. H_2O_2 -oxidation generated a nanoporous coating, and subsequent heat treatment above 500°C resulted in anatase formation. Tests using photo-assisted degradation of rhodamine B showed that prior to heat treatment, an initially high photocatalytic activity (PCA) of H_2O_2 -oxidized substrates decayed significantly with repeated testing. Heat treating the samples at 600°C resulted in stable yet lower PCA. Addition of 3% H_2O_2 during the photo-assisted reaction led to a substantial increase in PCA due to synergetic effects at the surface and H_2O_2 photolysis, the effect being most notable for non-heat treated samples. Both heat treated and non-heat treated samples showed stable PCA through repeated tests with H_2O_2 -assisted photocatalysis, indicating that the combination of H_2O_2 -oxidized titania films, UV light and added H_2O_2 can improve efficiency of these photocatalytic surfaces.

Highlights

- Ti and Ti64 substrates were surface modified by H_2O_2 -oxidation and heat treatments.
- TiO_2 /UV/ H_2O_2 synergy effects significantly boosted rhodamine B degradation.
- 3% H_2O_2 addition increased stability over repeated use.

Keywords

TiO₂; Heat treatment; Photocatalysis; Hydrogen peroxide photolysis; synergy

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1. Introduction

Biomedical implants and devices constructed from titanium and its alloys have long been used in orthopedics and dentistry to replace hard tissue, and much attention has been drawn to surface modification and functionalization to further improve their biological performance [1,2]. With regards to quick bone apposition and eradication of adherent bacteria, a TiO₂ surface layer can offer a dual solution through its photo-induced superhydrophilic and antibacterial properties [3-8]. These features complement the already long track record of successful osseointegration and biocompatibility of titanium-based implants [9,10]. As a site-specific, non-antibiotic method, photocatalytic decontamination can also serve to reduce the spread of multiresistant bacteria [11].

TiO₂ is a wide band gap semiconductor that becomes photocatalytically active when irradiated with wavelengths shorter than 385 nm (anatase structure). The photon energy is then sufficient to excite an electron (e⁻) to the conduction band, leaving a hole (h⁺) behind in the valence band. For a successful photocatalytic reaction to take place, the electron-hole pair must then be separated and electron acceptors or donors must be present at the semiconductor surface to compete with recombination of the pair [12]. The photo-induced electron transfer is governed by the band energy positions, determining the reduction potential of e⁻ and oxidizing ability of h⁺, and the redox potential of adsorbed species [8]. On TiO₂, surface hydroxyl groups (OH⁻) and adsorbed water can effectively trap holes to generate hydroxyl radicals (•OH), while adsorbed oxygen can trap electrons to form superoxide anions (O₂⁻). Holes and •OH are highly reactive species and primarily responsible for the oxidation of organic compounds such as bacteria on the surface [13]. TiO₂ photocatalysis has been widely researched since the 1970s, and applications of range from self-cleaning glass to decontamination of air, wastewater, and a variety of biomedical surfaces [5,14].

One application of particular interest is percutaneous (skin penetrating) implants and devices, where environmental exposure makes the surface susceptible for infection, but also

accessible for UV irradiation. As an *in-situ* photocatalytic decontamination system, such a surface must be effective with a low UV dose as to protect host tissue, and also possess stable properties if repeated activation would be necessary. Previous work has shown that H₂O₂-oxidation followed by hot water aging of commercially pure Ti (cpTi) and Ti-6Al-4V (Ti64) is a simple and cost effective way to obtain homogeneous, nanofeatured surfaces with both apatite forming abilities, aiding osseointegration, and enhanced photocatalytic activity (PCA). This has been attributed to an abundance of surface hydroxyl groups and high specific surface area [15-17]. However, poor crystallinity of such TiO₂ layers may limit their PCA, and while subsequent heat treatments may improve the crystallinity, such treatments eradicate beneficial hydroxyl groups and reduce the specific surface area [18,19]. Direct H₂O₂-oxidation of Ti is also known to produce a Ti-peroxy gel with associated superoxide radicals (Ti(IV)O₂⁻) [20-22]. These pre-existing radicals have been shown to contribute to initial activity against organic contaminants, but will inevitably be consumed [19]. Adding H₂O₂ during the photocatalytic reaction, on the other hand, has the potential benefit of enhancing efficiency by reducing electron-hole recombination [23] and producing hydroxyl radicals directly through UV absorption (photolysis) [24,25]. Due to these varied modes of action, it is of interest to compare the photo-induced degradation capacity of H₂O₂-oxidized Ti surfaces prior to and after heat treatment, and evaluate which surface or system would serve best in a biomedical setting, where repeated activation is necessary and UV dose should be restricted.

This study thus explores the combinatory effects of H₂O₂-oxidation, heat treatments and H₂O₂-assisted photocatalysis on titanium substrates. The aim is to screen titania films and obtain a high, sustained PCA, which could be applicable for future biomedical surfaces where on-demand antibacterial properties are of importance.

2. Materials and Methods

Samples of cpTi (grade 2) and Ti64 (grade 5) were manufactured as discs measuring 9 mm in diameter and 1 mm in thickness. Ti64 samples were cut from electron beam melted (EBM) rods (Arcam AB, Mölndal, Sweden), while cpTi samples were cut from wrought stock rods. Samples were ultrasonically cleaned in acetone, ethanol and double distilled water (ddH₂O) for 15 min each prior to further treatment. After drying in air, samples were subjected to oxidation in 30 mass% H₂O₂ solution (PERDOGEN™, Sigma-Aldrich, St. Louis, MO, USA) at 80 °C, aging in ddH₂O at 80 °C, and 1 h heat treatment in air according to Table 1. Times and temperature for hydrothermal treatment, as well as appropriate heat treatment temperatures, were based on previous work [17] and findings in the literature [19,26,27]. Each batch of samples contained at least four identical specimens.

Surface characterization was performed using the in-lens detector in a LEO 1550 scanning electron microscope (SEM; Zeiss, Oberkochen, Germany), and a Siemens D5000 grazing incidence X-ray diffractometer (GI-XRD; Bruker AXS GmbH, Karlsruhe, Germany) with the incident beam set to 1° and scanning 2 θ from 20° to 60° at a rate of 0.0125°/s.

PCA was quantified by measuring the degradation rate of the model substance rhodamine B in presence of UV illumination, titania samples and/or H₂O₂. Rhodamine B is an organic dye commonly used to evaluate photocatalytic materials, as reactive oxygen species (ROS) generated from the process degrade the dye molecules and the loss of color is easily monitored by UV/Vis absorption [28]. More efficient surfaces or systems, producing more ROS with the same amount of input energy (UV light), can thus be identified. Automated stepwise absorbance measurements of the solutions were made at 554 nm every 5 min using a UV/Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). For each run, 2.5 mL rhodamine B solution (5 μ M) was placed in a flat-bottomed quartz cuvette with 1 cm optical path length. The cuvette was covered with a UV

transparent glass lid to avoid evaporation and fitted with a small well enabling magnetic stirring for homogenization and continuous oxygen supply for the duration of each test. The UV light source (UV LED NCSU033B, Nichia, Japan) was incorporated in the spectrophotometer and irradiated samples and solutions with pulsing (100 Hz) light at 365 ± 10 nm, with a measured average intensity of 6.7 mW/cm^2 reaching the titania samples. The UV lamp was automatically blacked out during absorbance measurements. PCA (UV/TiO₂) and H₂O₂ assisted PCA (UV/H₂O₂/TiO₂) measurements were made in presence of four titania specimens placed at the bottom of the cuvette, while photolytic activity (PA; UV/H₂O₂) was measured in absence of titania specimens. PA and H₂O₂ assisted PCA measurements were performed by replacing 0.2 mL of rhodamine B with 30 mass% H₂O₂, resulting in a solution containing < 3 mass% H₂O₂ (0.79 M). Concentrations of H₂O₂ below 3% are routinely used in the oral cavity as a disinfectant, and moderate application is considered safe for human use. For repeated measurements of certain samples, to investigate if the PCA was decaying, discs were rinsed with ddH₂O, dried in air and re-tested at 24 h intervals.

Table 1 Preparation history of each sample batch

Batch id	Substrate material	t_1 : H ₂ O ₂ (h)	t_2 : H ₂ O (h)	HT temp. (°C)
1	cpTi	0	0	400
2	cpTi	0	0	500
3	cpTi	0	0	600
4	cpTi	24	0	400
5	cpTi	24	0	500
6	cpTi	24	0	600
7	cpTi	24	72	400
8	cpTi	24	72	500
9	cpTi	24	72	600
10	Ti64	0	0	400
11	Ti64	0	0	500
12	Ti64	0	0	600
13	Ti64	24	0	400
14	Ti64	24	0	500
15	Ti64	24	0	600
16	Ti64	24	72	400
17	Ti64	24	72	500
18	Ti64	24	72	600

19	Ti64	24	72	-
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3. Results

In Fig. 1, repeated measurements of photo-assisted rhodamine B degradation in presence of Ti64 samples after H_2O_2 oxidation and hot water aging for 24 h and 72 h, respectively (id 19, no heat treatment), is shown. The four consecutive curves thus represent the degradation power of the same samples when run multiple times. The degradation rate of rhodamine B can be approximated as a first order reaction, allowing quantification of PCA via the reaction rate constant in the equation $\ln(C/C_0) = -kt$, where C/C_0 expresses the normalized concentration, k is the reaction rate constant and t the reaction time [28]. The insert in Fig. 1 shows rate constant k for each of the four repetitive runs, which were performed at 24 h intervals. These experiments showed that after the first run, PCA was reduced to approximately one tenth of its initial value.

Fig. 1. Repetitive PCA measurements on non heat treated H_2O_2 -oxidized and hot water aged samples (id 19). Insert showing the quantification of each curve in terms of rate constant k , indicating decay in PCA with repeated use of the same samples.

PCA of samples after heat treatments are shown in Fig. 2, where rate constant k is given as a function of heat treatment temperature. Each point represents the combined value produced by four specimens during each respective run, and obtained regression coefficients (R^2) from the determination of the rate constants are given in Table 2. Samples heat treated at 400 °C and 500 °C generally performed in the same range as non-heat treated samples after stabilization (see Fig. 1). Samples pre-oxidized in H_2O_2 showed an increase in PCA with increased heat treatment temperature, while direct heat treatment without pre-oxidation led to little or no increased PCA. Highest PCA, comparable to the initial value of non-heat treated samples with H_2O_2 -oxidation, was noted for cpTi samples after full treatment, i.e. H_2O_2 -oxidation, hot water aging and 600 °C heat treatment (id 9). Ti64 samples followed a similar pattern as the cpTi samples, but presented slightly less PCA. Repeated measurements showed equal or slightly decreasing values after several runs.

Fig. 2. PCA of heat treated samples given as a function of heat treatment temperature. Legend indicates substrate material and pre-oxidation times (h): $H_2O_2 + H_2O$

Table 2 Rate constant k and coefficient of determination R^2 of heat treated samples. Sample name indicating substrate material and pre-oxidation times (h): $H_2O_2+H_2O$

Sample	HT temp (°C)	k (10^{-3} min^{-1})	R^2
cpTi 0+0	400	0.03	0.923
	500	0.02	0.893
	600	0.04	0.832
cpTi 24+0	400	0.07	0.980
	500	0.21	0.993
	600	3.77	0.998
cpTi 24+72	400	0.40	0.987
	500	0.65	0.990
	600	4.59	0.998
Ti64 0+0	400	0.07	0.976
	500	0.07	0.957
	600	0.14	0.978
Ti64 24+0	400	0.08	0.961
	500	1.03	0.992
	600	1.94	0.988
Ti64 24+72	400	0.11	0.981
	500	0.63	0.985
	600	2.80	0.998

Surface layer crystallinity of heat treated samples are shown in Fig. 3, where GI-XRD reveals TiO_2 peaks for samples treated at 500 °C and 600 °C. Samples subjected only to heat treatments (id 1, 2, 3, 10, 11 and 12) and H_2O_2 -oxidized samples with or without hot water aging treated at 400 °C (id 4, 7, 13 and 16), showed no clear TiO_2 peaks. A progressive transition from anatase to rutile is known to occur at temperatures around 600 °C and above [27,29,30]. This was visible for Ti64 with the most pronounced rutile peak around 27° (110), produced after H_2O_2 -oxidation and direct heat treatment at 600 °C (id 15). However, only anatase was found on cpTi. In all cases, Ti peaks originating from the substrates were visible.

SEM images in Fig. 4 show selected surface morphologies after H_2O_2 -oxidation, hot water aging and/or heat treatment. Scattered crystals were nucleated on Ti64 without H_2O_2 -oxidation after

600 °C heat treatment (Fig. 4b); however the layer was too thin or amorphous to generate a GI-XRD signal. Fig. 4c through Fig. 4f show the nanoporous, sponge like surface layer generated on Ti by 24 h H_2O_2 -oxidation, and that surface morphology was generally maintained throughout hot water aging and/or heat treatment up to 600 °C.

Fig. 3. GI-XRD spectrums of heat treated samples. Numbers on left hand side indicates prior oxidation and aging times (h): $H_2O_2 + H_2O$. Heat treatment temperatures are indicated on the right

Fig. 4. SEM images of surface morphologies; **a)** Ti64 HT400 (id 10), **b)** Ti64 HT600 (id 12), **c)** Ti64 24+72 HT400 (id 16), **d)** Ti64 24+72 HT600 (id 18) **e)** cpTi 24+0 HT400 (id 4) **d)** cpTi 24+0 HT600 (id 6)

Fig. 5 shows collected measurements of rhodamine B degradation under various conditions, including photolysis (UV/H₂O₂) and H₂O₂ assisted photocatalysis (UV/H₂O₂/TiO₂), using non-heat treated samples (id 19). Corresponding rate constants are shown according to increasing value in Fig. 5 insert. A five-fold increase in activity was noted when comparing H₂O₂ assisted PCA with regular PCA on identical samples. A considerable degradation rate was also seen under direct photolysis of H₂O₂. A small rate constant increase occurred in the H₂O₂/TiO₂ system compared to H₂O₂ and TiO₂ alone, suggesting an autocatalytic reaction at the surface.

Fig. 5. Normalized degradation curves of rhodamine B under varying conditions. Legend indicates assisting entities during each measurement. Insert showing rate constant k derived from each curve, according to increasing value: $\text{TiO}_2 < \text{H}_2\text{O}_2 < \text{H}_2\text{O}_2/\text{TiO}_2 < \text{UV} < \text{UV}/\text{TiO}_2 < \text{UV}/\text{H}_2\text{O}_2 < \text{UV}/\text{H}_2\text{O}_2/\text{TiO}_2$. Non-heat treated TiO_2 surfaces (id 19) were used.

Repeated measurements of H_2O_2 assisted PCA, performed at 24 h intervals on H_2O_2 -oxidized and hot water aged samples (id 19), as well as their heat treated counterparts (id 18), are shown in Fig. 6. The system containing non-heat treated samples showed a higher activity throughout repeated tests. Rate constants k (indicated in Fig. 6) revealed a slight, gradual decline in both systems.

Fig. 6. Repetitive tests of H_2O_2 assisted photodegradation of rhodamine B in presence of titania films. Rate constant k (10^{-3} min^{-1}) is indicated for each run

4. Discussion

Providing functional materials and efficient methods for decontaminating biomedical implants and devices is a key issue to ensure their long-term service and stability [31]. Systems penetrating soft tissue, such as bone anchored prostheses or dental implants, are particularly susceptible to infection due to high exposure to bacteria from the environment, and the application of antibiotics for combatting these infections in such cases may not be sufficient [11,32]. Reducing the general use of antibiotics to treat infection has also been a long standing call to limit bacterial resistance [33]. In applying the photocatalytic bactericidal properties of titania, an on-demand feature for decontamination can be added to biomedical surfaces, and is of particular interest for percutaneous implants and devices. A number of studies have been devoted to this matter and have shown promising results [4,11,34-37], but further research is needed to improve efficiency and avoid adverse effects of UV light on healthy tissue, etc.

In this study, stability issues in PCA of H_2O_2 -oxidized Ti were addressed by means of heat treatments and assisting photocatalysis with added H_2O_2 . By using a chemical oxidant such as H_2O_2 to modify the surface of Ti, a nanofeatured and homogeneous coating, as can be observed in Fig. 4, can be obtained on devices with complex shapes at low cost. H_2O_2 -oxidized and hot water aged Ti has previously been shown to generate an abundance of Ti-OH groups at the surface, contributing to the ability of apatite formation [15,26]. Such surface groups also benefit the photocatalytic reaction by increasing oxygen adsorption and preventing electron-hole recombination [38-40]. However, the sudden drop in PCA after repeated use, as seen in Fig.1, suggests a dramatic decrease in the production of radical species at the surface, or rather, a rapid consumption of radical species already present. As reported by Tengvall et al. [20,22], Ti and H_2O_2 interactions produce a Ti-peroxy gel containing superoxide radicals coordinated to Ti(IV), and as observed by Wu [19], these

radical species contribute to initial activity but will gradually be consumed and lead to a decline in PCA, limiting the practical use of such coatings for efficient and repeated decontamination.

Heat treating the H₂O₂-oxidized samples at 600 °C led to a certain degree of crystallization and anatase formation, without significantly affecting the surface morphology. Although more stable with repeated testing, the PCA of these samples (see Fig. 2) were generally found to be inferior to the initial value of non-heat treated samples, which had a rate constant $k = 4.8 \times 10^{-3} \text{ min}^{-1}$. Further, the results showed lower values for Ti64 compared with cpTi after heat treatments, which could be due to partial rutile formation on Ti64, as well as formation of other alloying element oxides, such as Al₂O₃ and V₂O₅, known to reduce PCA [41]. The near linear trend in PCA with temperature seen for sample Ti64 24+0 in Fig. 2, compared to the sharper increase at 600°C for other H₂O₂-oxidized samples, is likely due to a transformation from anatase to rutile, as visible in the GI-XRD data (Fig. 3). The anatase-to-rutile ratio has been shown important with regards to PCA and is principally related to surface adsorption of water and hydroxyl groups, where anatase is more active [42].

At temperatures above 300 °C, Ti-OH groups and radical species stabilized in Ti-H₂O₂ complexes are effectively decomposed and anatase is reported to precipitate from the amorphous Ti-peroxy gel [21]. Crystallization of this layer also reduces its solubility in water, limiting release of any surface bound peroxides. This supports the theory that heat treated samples exhibit a larger degree of heterogeneous photocatalysis on crystalline anatase, whereas activity on H₂O₂-oxidized samples depend more on photo-induced activation of radical species at the surface and slow release of surface bound peroxides.

The increased activity when assisting photocatalysis with H₂O₂, seen in Fig. 5, can be explained by: (I) its superior efficiency compared to O₂ in scavenging TiO₂ conduction band electrons to produce additional •OH radicals, thereby also limiting electron-hole recombination [23,43]; and (II) its potential to undergo direct photolysis under UV light to generate two •OH

radicals [24,25]. The action of H_2O_2 photolysis has recently also been shown to be effective against oral bacteria *in vivo* using visible light [44], extending the potential for clinical applications. The combinatory effects of H_2O_2 assisted photocatalysis through actions (I) and (II), and the maintained high activity made the system superior to unassisted photocatalysis or photolysis alone. This has similarly been demonstrated in applications for wastewater treatment [43]. Non-heat treated surfaces were also found to outperform their heat treated counterparts through repeated tests with added H_2O_2 (Fig. 6). In the event of photocatalysis, this could be explained by an increased affinity of electron scavengers O_2 and H_2O_2 to a surface with abundant Ti-OH groups and increased surface area, rather than high crystallinity [17]. Prolonged UV irradiation of amorphous Ti-oxide has also been reported to rearrange surface bound OH groups and increase crystallinity [45], which could potentially increase the occurrence of heterogeneous photocatalysis over time. In this instance however, the maintained high activity is attributed to abovementioned actions and, on non-heat treated surfaces, the possibility of Ti-peroxy complexes to be restored when adding 3% H_2O_2 for the assisted reaction [46]. The suspected photocatalyst deactivation seen in Fig. 6 (gradual decrease of rate constant k) is a known issue [8], and is believed to stem partly from rhodamine B adsorption or accumulation of intermediates on the surface, blocking active sites, as well as a decline in Ti-peroxy complex restoration. A comparatively high activity was however maintained through several runs, making the system suitable for repeated use.

5. Conclusions

In summary, titanium substrates (cpTi and Ti64) were surface modified by means of a simple and straightforward H_2O_2 -oxidation technique, followed by aging in H_2O and/or heat treatment up to 600 °C to achieve titania films with high, sustained PCA. Heat treatments above 500 °C led to crystallization and anatase formation on samples pre-oxidized in H_2O_2 , coupled with a gradual increase in PCA. A substantial increase, however, was first noted when assisting the photocatalytic reaction with an added 3% H_2O_2 , a concentration applicable for clinical use. The effect was largely maintained through repeated tests, and highest for H_2O_2 -oxidized, non-heat treated surfaces. By combining the beneficial surface properties obtained by H_2O_2 -oxidation of Ti, with the oxidative power of H_2O_2 under UV as well as its contributory effects to the photocatalytic reaction, an effective system for on-demand organic degradation was devised. This system can potentially be applied to biomedical Ti surfaces penetrating soft tissues, where repeated decontamination is essential to avoid severe infection.

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