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A Synergistic Effect of Albumin and H_2O_2 Accelerates Corrosion of Ti6Al4V

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Abstract

The synergistic effect of albumin and H_2O_2 on corrosion of titanium alloy Ti6Al4V in physiological saline was investigated with long-term immersion tests and electrochemical methods. It was found that in the presence of both albumin and H_2O_2, the rate of metal release in immersion tests was far higher than in the presence of either species alone. Electrochemical polarisation curves and potentiostatic tests showed that H_2O_2 increased both the rates of the anodic and cathodic reactions, while albumin significantly decreased the rate of the cathodic reaction and slightly decreased the rate of the anodic reaction. The synergistic effect of albumin and H_2O_2 during immersion tests was attributed to the effect of adsorption of albumin in lowering the rate of the cathodic reaction and thus lowering the open circuit potential into the active region of titanium where complexation by H_2O_2 increased the corrosion rate. The corrosion attack was found to be greater in the β-phase of the alloy. The findings suggest that current standard tests in physiological or phosphate-buffered saline may underestimate the rate of corrosion in the peri-implant environment, in which albumin is the predominant protein, and reactive oxygen species such as H_2O_2 can occur as a result of inflammatory reactions in response to surgery, infection, or implant corrosion products.

Introduction

Standard tests to study corrosion properties of biomedical alloys are typically carried out in solutions such as phosphate-buffered saline (PBS) [1-3] or physiological saline (0.9% NaCl) [4, 5]. However, the peri-implant environment contains a large range of biomolecules including proteins such as albumin [6] and products of cellular physiology such as reactive oxygen species (ROS) which includes hydrogen peroxide (H_2O_2) [7, 8]. The effects of these species have been extensively investigated individually [9-20], but never in combination. In this paper, we show that a combination of albumin and H_2O_2 can lead to much higher levels of corrosion of Ti6Al4V than either species alone, indicating the importance of developing tests that provide a more realistic simulation of the composition of the peri-implant environment.

Ti alloys are routinely used for biomedical applications because they have good biocompatibility, suitable mechanical properties and corrosion resistance [21]. However corrosion-related failures of implanted Ti devices have been recognised to occur *in-vivo* [22-24]; significantly elevated Ti levels have been detected in humans or animals with Ti implants [25-30]; and the analyses of peri-implant tissues and more distant tissues retrieved from patients with Ti implants have identified the presence of Ti species [31-36]. The biological consequences of the released Ti corrosion products are reported to include immunological responses leading to chronic inflammation around the implant which may ultimately lead to implant loss [22, 23, 32].

Ti implants are exposed to complex physiological environments that contain salts, lipids, proteins as well as living cells and/or bacteria which themselves can generate a variety of biomolecules in response to external stimulation [9, 37]. Local inflammation can occur around implants as a result of infection or indeed a result of the presence of debris derived

from the implant itself [22, 23]. Elemental analysis of oral mucosa surrounding Ti dental implants has demonstrated increased Ti release from the implant surfaces in inflamed sites when compared with healthy tissues although the exact mechanisms underpinning the observation were not determined [34]. During inflammation, levels of H_2O_2 may become elevated being produced directly by bacteria (at levels which can exceed 5mM) or by immune cells which have migrated to the inflamed site [7, 8]. H_2O_2 has been demonstrated to decrease the corrosion resistance of Ti implants [9-13] leading to surface roughening [13, 38] and the selective dissolution of the β phase of Ti6Al4V [39, 40]. This has been attributed to the complexation reaction between Ti and H_2O_2, and the structure of the corrosion product has been proposed as a strongly hydrated Ti(IV)-H_2O_2 complex [41-44].

In addition following implantation, proteins quickly adsorb on the surface of an implant (e.g. Ti [45] and stainless steel [46]). Albumin is the most abundant protein found in blood (~4%) and in the extracellular environment, and its influence on the corrosion behaviour of metallic implants (including Ti) has been extensively reported [6, 14, 45-48]. However, the effect of albumin on the corrosion rate of Ti alloys is still unclear. Conflictingly, the presence of albumin has been reported to either decrease the corrosion rate of Ti alloys [15-17, 19, 49] or increase it [16, 18, 20] or have no effect [14, 50-52]. It is generally accepted that the addition of albumin decreases the cathodic reaction on Ti alloys, indicating that albumin serves as a cathodic inhibitor [14, 49, 51].

There are currently two papers where albumin and H_2O_2 have both been mentioned in relation to Ti corrosion: in one paper on the effect of albumin, it was noted that H_2O_2 is an intermediate species in the oxygen reduction reaction [16], and in another, it was noted that adsorption of albumin is higher on Ti surfaces previously exposed to H_2O_2 [53]. However, there has been no systematic study of the synergistic interaction between H_2O_2 and albumin on Ti corrosion, and all studies to date have studied the effects of the two species

independently. Accordingly in this paper we test the null hypothesis that the effects of H_2O_2 and albumin on the corrosion of Ti6Al4V are unaltered by the combination of the two species.

1 Materials and Methods

1.1 Ti sample preparation

Ti6Al4V alloy (ASTM Grade 5) is a high strength α/β alloy commonly used in orthopaedic, craniofacial and dental applications. Ti6Al4V discs (14 mm diameter and 1.2 mm thickness) were commercially sourced and machined by the supplier (Titanium Products Ltd, Solihull, UK).

For immersion tests, samples were mirror-polished on both sides according to a previously identified protocol [54], by first grinding with MD-Piano abrasive cloths (Struers, Ballerup, Denmark) in deionised water (Millipore, 18 MΩ), followed by MD-Largo (Struers) with a 9 µm diamond suspension. Finally the samples were polished with a MD-Chem polishing cloth (Struers) using 0.04 µm OP-S Colloidal Silica suspension (Struers). All samples were thoroughly cleaned sequentially in acetone, ethanol, and deionised water, using ultrasonic agitation for 10 min at each stage. Specimens were finally dried in an air stream prior to use in further experiments.

For electrochemical tests, Ti discs were mounted in VARI-SET cold mounting acrylic (MetPrep Ltd, Coventry, UK) and used as the working electrode (WE, working area 1.5 cm^2). To obtain a good reproducibility in electrochemical experiments, the time between polishing and electrochemical measurements was controlled: after final polishing with an MD-Chem polish cloth, the samples were immediately cleaned with deionised water, then dried in an air stream and left in open air for 5 min before immersing in test solutions.

1.2 Solutions

For immersion tests, H_2O_2 (30% w/v, 30 g H_2O_2 in 100 mL solution, BDH, England, UK) and bovine serum albumin (≥ 98% (agarose gel electrophoresis) lyophilised powder, Sigma-Aldrich, Dorset, UK) was added to physiological saline (0.9% NaCl (Sigma-Aldrich, Dorset, UK), pH 6.7-7, 0.15 M). To minimise contamination with elements of interest for subsequent mass spectrometry measurements, ultrapure water (Gibco®, life technologies™, Paisley, UK) were used throughout for immersion tests.

For electrochemical tests, similar solutions were made using deionised water (Millipore, 18 MΩ).

1.3 Immersion tests

All prepared mirror-polished Ti6Al4V samples were immersed in 2 mL of test solution in a 30 mL universal tube (transparent polystyrene with conical bottom) and sealed to prevent evaporation. For each experimental condition, three Ti samples were immersed and incubated at 37 °C. Samples were gently agitated on an A500 orbital mixer (Denley, UK) for 1 h per day. For each sample, the test medium was retrieved and replaced with new solutions following 7 days. Following 4 weeks of immersion, the retrieved solutions for each sample in the presence of different level of H_2O_2 were pooled together for elemental concentration measurements.

The surfaces of the Ti6Al4V discs were characterised by scanning electron microscopy (SEM, JEOL 7000 (Jeol Ltd., Tokyo, Japan) using an accelerating voltage of 20 kV, a beam current of ~70 μA) and energy dispersive X-ray spectroscopy (EDX, detector model: 7558, collecting window: ATW 2; acquisition time: 60 s; quantification method: standardless; Oxford

Instrument, UK). It was confirmed by EDX that α phase contains 6±0.7 wt% Al and 2.5±0.2 wt% V and β phase contains 4.2±0.4 wt% Al and 9±1.8 wt% V.

Test solutions from each sample were syringe-filtered (Acrodisc® 32 mm syringe filter with 0.45 µm Supor® membrane, Pall Newquay, UK) and refrigerated at 4 °C prior to elemental concentration measurement. Elemental Ti, Al and V concentrations in the immersion solutions were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500ce ICP-MS, Agilent Technologies, CA, USA) where the detectable limit for Ti, Al and V was 0.2 ppb (ng/mL). One-way analyses of variance (ANOVA) and post-hoc Tukey tests were conducted to identify statistical differences (α=0.05) between ICP-MS quantifications of Ti, Al or V in immersion solutions containing varying concentrations of H_2O_2 or varying concentrations of albumin.

1.4 Electrochemical tests

A standard three-electrode cell with reference electrode (RE), counter electrode (CE) and working electrode (WE) was used. The CE was a Pt mesh (working area ~4 cm^2) and the RE was a commercial saturated calomel electrode (SCE). The potential was controlled with a potentiostat (Model 1408, ACM Instruments, UK). The electrochemical cell was immersed in a water bath maintained at 37±1 °C.

For polarisation curves, the open circuit potential (OCP) was measured for 1 h and then anodic and cathodic polarisation curves were performed. Anodic polarisation curves were measured by sweeping the potential from -50 mV below the OCP to 1200 mV vs. SCE whilst the cathodic polarisation curves were measured separately by sweeping the potential from 50 mV above the OCP to -1400 mV vs. SCE at a rate of 1 mV/s. Potentiostatic measurements were also performed following 1 h immersion at OCP. In addition, OCP of mirror-polished

Ti6Al4V in physiological saline with and without addition of H_2O_2 and/or albumin were all monitored for 24 h. All electrochemical tests were performed at least twice for each condition, using a freshly polished sample and fresh solution in each case.

The surface morphology of samples was examined using both SEM and atomic force microscopy (AFM) in contact mode (Digital Instruments, Veeco Metrology Group; Model No.: MMAFMLN; California, USA) before and after the potentiostatic measurement in 10% H_2O_2. To easily locate the same region for comparison before and after exposure, one region of the mirror-polished sample was identified by an indentation mark (Vickers hardness test, load: 300 g, MVK-H1 hardness testing machine, Mitutoyo, Japan) which was then characterised by SEM. The carbon layer generated on the surface from SEM was removed by quickly (~10 s) polishing the sample on an MD-Chem polish cloth prior to cleaning as described above so that it would not affect the electrochemical experiments.

2 Results

2.1 Effect of H_2O_2 on corrosion of Ti6Al4V

Figure 1 demonstrates that increased concentrations of Ti, Al and V were released from Ti6Al4V with increasing concentration of H_2O_2. For each element a one-way ANOVA demonstrated a significant impact of H_2O_2 concentration on detected element release ($p<0.001$). More V was released than Al, especially following immersion in lower concentrations of H_2O_2. Ti, Al and V levels in the control solutions (no Ti disc) were negligible. Following Ti immersion, yellow precipitates were observed in the solution in 10% H_2O_2 probably due to detachment of the surface corrosion product (Ti-H_2O_2 complex [43]). Therefore, the Ti concentrations released from Ti6Al4V were not measured in 10% H_2O_2 since the solid products would not be detected with the method used.

Figure 2 shows micrographs of the surface morphology of Ti6Al4V after immersion tests in physiological saline with different levels of H_2O_2. Figure 2a and Figure 2b show that in the absence of H_2O_2 the surface was unattacked, and appeared similar to the as-polished surface. With increasing H_2O_2 concentration the surfaces show signs of corrosion attack, particularly on the β phase, as well as formation of corrosion products on the surface.

The preferential attack of the β phase can be clearly seen in Figure 2k and Figure 2l following potentiostatic polarisation of Ti6Al4V at 600 mV for 30 min at at 37 °C. The BSE image shows clear attack at the edge of β phase, while AFM shows that the whole β phase area was attacked to a depth of ~50 nm.

Figure 3a shows that the OCP increases with time following immersion and the values were almost 300 mV higher in the presence of H_2O_2. Figure 3b shows cathodic polarisation curves measured in physiological saline with and without H_2O_2. A cathodic current density plateau

was observed for Ti6Al4V under very negative potentials (less than -1 V vs. SCE) due to limited oxygen diffusion. With the addition of H_2O_2, the cathodic current densities were increased due to the reduction reaction of H_2O_2. It can also be seen that the cathodic current densities increased with increasing concentration of H_2O_2 and the value of OCP moved to more positive values in the presence of H_2O_2, which is consistent with the OCP measurements in Figure 3a.

It can be seen from the anodic polarisation curves (Figure 3c) that Ti6Al4V showed passive behaviour in physiological saline and no significant change of anodic current density was observed up to a potential of 1200 mV vs. SCE. In the presence of H_2O_2, the anodic current densities became higher compared with those measured in physiological saline without H_2O_2. The anodic current densities increased with increasing concentration of H_2O_2. Furthermore the anodic current densities of Ti6Al4V in the presence of H_2O_2 were slightly increased with increasing potential until an abrupt increase at 600-800 mV vs. SCE was observed, possibly due to oxygen evolution.

2.2 Effect of albumin on corrosion of Ti6Al4V

Figure 4a demonstrates the OCP as a function of time for mirror-polished Ti6Al4V. The OCP increased with time and was almost 300 mV lower in the presence of albumin. The OCP of Ti6Al4V did not show significant differences in solutions containing different levels of albumin. Figure 4b shows that albumin addition significantly inhibited the cathodic reaction when compared with the cathodic reaction in physiological saline. The decreased cathodic current densities did not show significant differences in the solutions containing different levels of albumin. In addition, the OCP of Ti6Al4V moved to more negative values in the presence of albumin, which is consistent with OCP measurements in Figure 4a. The anodic current density was observed to be higher in the presence of albumin. This is possibly due to

the suppressed cathodic reaction. A particularly sharp active peak was found in 4% albumin (Figure 4d).

2.3 Effect of combination of H_2O_2 and albumin on corrosion of Ti6Al4V

Figure 5 shows the concentrations of Ti, Al and V released from mirror-polished Ti6Al4V in the absence and presence of H_2O_2 and albumin. Ti, Al and V levels in the control (without a Ti6Al4V disc) solutions were negligible, except for the Al level in the albumin control sample (15±10 ppb) (Figure 5) and it is possible that this was due to one contaminated sample. Of the three independent measurements, only one sample showed high Al level (26 ppb, 8 ppb and 10 ppb).

A significantly higher concentration of Ti was measured following immersion of the Ti samples in the presence of H_2O_2 when compared with H_2O_2-free solution ($p<0.01$) and similar to the 4 week immersion tests (see Figure 1), the amount of released V was greater than Al. In the presence of albumin, a small increase ($p<0.01$) in the measured Ti concentration (65±5 ppb) was observed when compared with the (albumin-free) physiological saline control. Immersion in mixed solutions of H_2O_2 and albumin resulted in a substantial increase in the release of Ti, Al and V ($p<0.001$ for each element), which increased with the increasing concentration of albumin in the mixed solutions. The concentration of Al measured in the mixed solutions was greater than that in the presence of H_2O_2 without albumin ($p<0.01$).

Figure 6 shows that the corrosion products on the Ti6Al4V surface appeared porous in the presence of H_2O_2 based on the secondary electron (SE) image, and the β phase appeared to be preferentially attacked based on the backscatter electron (BSE) image, which was similar to the observation following the 4 week immersion tests in H_2O_2 (Figure 2). Figure 6 also shows

that the surface of Ti6Al4V after immersion in albumin did not show a significant difference from the sample immersed in physiological saline alone. However, after immersion in the mixed solutions of H_2O_2 and albumin the surface of Ti6Al4V was relatively smooth with no obvious corrosion products and the β phase was preferentially attacked. Figure 7a shows the OCP as a function of time for mirror-polished Ti6Al4V in the absence and presence of H_2O_2 and albumin. The OCP was higher in the presence of H_2O_2 compared with (H_2O_2-free) physiological saline, and was lower after the addition of albumin. The OCP was lower in the presence of albumin compared with (albumin-free) physiological saline, and was higher after the addition of H_2O_2. The OCP of Ti6Al4V in the presence of mixed solutions of H_2O_2 and albumin was close to that in the physiological saline control. In addition, the OCP was not found to depend on the concentration of albumin in the mixed solutions.

Figure 7b shows cathodic polarisation curves for mirror-polished Ti6Al4V in the absence and presence of H_2O_2 and albumin. It can be seen that the addition of albumin decreased the cathodic current density in the presence of H_2O_2 alone, and the addition of H_2O_2 increased the cathodic current density in the presence of albumin alone.

For anodic polarisation curves (Figure 7c), Ti6Al4V exhibited higher anodic current densities in mixed solutions of H_2O_2 and albumin compared with physiological saline, and this appears to be mainly due to an enhanced anodic reaction. In addition, the anodic current densities of Ti6Al4V in mixed solutions were increased with an increasing concentration of albumin (Figure 7d), which is consistent with the metal release results (Figure 5). The anodic current density of Ti6Al4V showed an abrupt increase in the presence of mixed solutions at 600±80 mV vs. SCE, which was similar to that observed in H_2O_2 (see Section 3.1.3.1) and is possibly due to oxygen evolution.

Figure 8 illustrates the potentiostatic measurements of mirror-polished Ti6Al4V in physiological saline with addition of albumin and/or H_2O_2 at different potentials. The solution was stirred at ~1000 s intervals to ensure complete mixing. H_2O_2 and/or albumin were added just prior to stirring for some of the measurements. Figure 8a demonstrates that the cathodic steady state current densities of Ti6Al4V at -800 mV vs. SCE were decreased following addition of albumin at the 1st ~1000 s interval, consistent with the cathodic polarisation results (Figure 4b). It can also be seen that the cathodic steady state current densities in the absence and presence of albumin were increased after addition of H_2O_2 at the 2nd ~1000 s interval, consistent with the cathodic polarisation results in Figure 7b. In addition, the cathodic steady state current densities in H_2O_2-containing solution were decreased following addition of albumin at the 3rd ~1000 s interval, which is also consistent with the cathodic polarisation results (Figure 7b).

Figure 8b and Figure 8c show that the anodic steady state current densities of Ti6Al4V at both anodic potentials decreased after addition of albumin at the 1st ~1000 s interval. It has also been shown that at the 2nd ~1000 s interval, the anodic steady state current densities in the absence and presence of albumin were increased after addition of H_2O_2. In addition, at the 3rd ~1000 s interval, the anodic steady state current densities in the presence of H_2O_2 were also decreased after addition of albumin.

Figure 9 shows the OCP as a function of time for 24 h for mirror-polished Ti6Al4V in physiological saline with and without H_2O_2 and albumin. The solution was stirred at intervals to ensure complete mixing. H_2O_2 or albumin was added just prior to stirring at ~1 h or ~4 h for some of the measurements. At ~1 h following addition of H_2O_2, the OCP of Ti6Al4V shifted to a higher value when compared with the OCP in physiological saline alone. At ~4 h when albumin was added into the H_2O_2-containing solution, the OCP decreased and this was consistent with the 1 h OCP measurement (Figure 7a). At ~1 h following addition of

albumin, the OCP of Ti6Al4V decreased when compared with the OCP measured in physiological saline alone. Subsequently at ~4 h when H_2O_2 was added into the albumin-containing solution, the OCP of Ti6Al4V increased, which again was consistent with the 1 h OCP measurement (Figure 7a). After 24 h exposure, Ti6Al4V exhibited the highest OCP value in H_2O_2 and the lowest in albumin whilst the OCP in mixed solutions was close to that in physiological saline, which also agreed with the previous observations (see Figure 7a).

3 Discussion

3.1 Effect of H_2O_2 on corrosion of Ti6Al4V

Ti6Al4V is highly resistant to corrosion in physiological saline: it can be seen from Figure 1 that the concentrations of Ti, Al and V measured in the control solutions were negligible. However, the concentrations of released metal ions (Figure 1) and anodic current densities measured in polarisation curves (Figure 3) increased with the increasing concentration of H_2O_2, indicating decreased corrosion resistance of Ti6Al4V in the presence of H_2O_2, which is consistent with previous studies on Ti alloys [10-13, 55] and has been explained in terms of the complexation of Ti by H_2O_2 [41-44].

Since all experiments were carried out under naturally-aerated conditions, it is assumed that the cathodic reaction in physiological saline is oxygen reduction. However, in the presence of H_2O_2 the cathodic reaction is likely to be dominated by the reduction reaction of H_2O_2 since H_2O_2 is a strong oxidiser and has a high standard reduction potential ($E° = 1.54$ V vs. SCE) [56], leading to a higher cathodic current density and also resulting in a more positive OCP.

3.2 Effect of albumin on corrosion of Ti6Al4V

It can be seen that the presence of albumin significantly inhibits the cathodic reaction of Ti6Al4V (Figure 4 and Figure 8), resulting in a lower OCP (Figure 4), consistent with previous studies on Ti alloys [14, 51]. It has been proposed that albumin strongly adsorbs on the surfaces of metals including Ti by chemisorption through carboxylate/amino group or through electrostatic interactions, blocking electrochemical reactions [14, 45, 57].

In the current study, the anodic steady state current density under potentiostatic conditions decreased following addition of albumin (Figure 8), indicating that the anodic reaction was suppressed. Similar observation have been made on CP-Ti [14] and Ti6Al4V-ELI [16]. It is possible that the adsorption of albumin also inhibits the anodic reaction of Ti6Al4V, resulting in a lower anodic steady state current. Therefore, the observed higher net anodic current densities in polarisation curves in the presence of albumin (Figure 4) are more likely to be due to the suppressed cathodic reaction.

It is known that Ti may show an "active" peak at low potential. This has been attributed to the formation of Ti^{3+} [58]. A particularly sharp active peak was found in the presence of 4% albumin (Figure 7), which has not been previously reported. It should be noted that in previous studies [17, 19, 51], polarisation curves started from very low potentials (e.g. -1 V vs Ag/AgCl [51]), whereas in the current study, anodic and cathodic polarisation tests were conducted separately to reduce the effect of prior cathodic polarisation on anodic polarisation and vice versa. The history of cathodic reaction may have an influence on anodic behaviour of Ti6Al4V during the polarisation tests.

A small increase in Ti release from Ti6Al4V was observed in the presence of albumin after the immersion test (Figure 5). However, the presence of albumin inhibited the cathodic and anodic reactions in physiological saline. Similar apparently contradictory results can be found in the work of Padilla and Bronson [16] and Karimi and Alfantazi [17-20]. The most likely explanation is that the lower OCP brought about by the suppressed cathodic reaction may drive the dissolution of Ti6Al4V into the "active" region, so the presence of albumin increases the corrosion rate of Ti6Al4V at the open circuit potential.

3.3 Effect of the combination of albumin and H_2O_2 on corrosion of Ti6Al4V

It can be seen from Figure 7 that the cathodic current density is lower in the presence of both H_2O_2 and albumin when compared with those in H_2O_2 alone, suggesting that the presence of albumin inhibits the cathodic reaction of Ti6Al4V in H_2O_2-containing solutions. In polarisation curves (Figure 7) higher net anodic current densities were observed in the presence of mixed solutions when compared with those in the presence of H_2O_2 alone: this is likely to be due to the suppressed cathodic reaction. Potentiostatic studies showed that the anodic steady state current densities (Figure 8) in the presence of H_2O_2 were decreased after addition of albumin, indicating that the presence of albumin suppressed anodic reaction of Ti6Al4V in H_2O_2-containing solutions.

A considerably higher rate of metal release from Ti6Al4V was observed in mixed solutions compared with that in either albumin or H_2O_2 alone after immersion tests (Figure 5), which has not previously been reported. This attack was found to be greater in the β-phase of the alloy (Figure 6). The most likely explanation is that the cathodic reaction is suppressed by the addition of albumin, taking the potential of Ti6Al4V into the active region, thereby increasing the rate of corrosion. Figure 10 shows a diagram to explain this hypothesis. The anodic and cathodic reactions in the absence of H_2O_2 and albumin are shown by the thin black lines. The anodic and cathodic reactions intersect in the passive region at point A. Addition of albumin to physiological saline (thick green lines) slightly decreases the anodic reaction and greatly inhibits the cathodic reaction; the anodic and cathodic curves intersect in the active region below the peak at point B, giving a slightly higher corrosion rate (Figure 5) and lower OCP (Figure 9). Addition of H_2O_2 to physiological saline (thin dashed blue lines) substantially increases both the anodic and cathodic reactions, with the intersection at point C in the passive region, giving a higher OCP (Figure 9) and higher corrosion rate (Figure 5).

With addition of both H_2O_2 and albumin to physiological saline (thick dashed red lines), the anodic reaction is slightly lower than with H_2O_2 alone, and the cathodic reaction is also lower than that with H_2O_2 alone, but the lines intersect in the active region (point D) so the OCP is similar to that physiological saline in the absence of H_2O_2 and albumin (Figure 9), but the corrosion rate is much higher (Figure 5).

The results presented in this paper show that addition of both H_2O_2 and albumin to physiological saline leads to a far higher rate of corrosion of Ti6Al4V than that in physiological saline alone. Albumin is the most common protein in the peri-implant environment and ROS such as H_2O_2 are produced in the presence of inflammation reaction [7, 8], which are common after surgery, or in response to infection or corrosion products. However, current standard tests for biomedical alloys use saline solutions e.g. PBS and 0.9% NaCl [1-5], in which far lower corrosion rates are observed. The considerable increase in corrosion rate observed in the current work in the presence of both albumin and H_2O_2 suggests that current standard tests may underestimate the true rate of corrosion of implants in the body, and that testing should take place in solutions that more closely simulate the peri-implant environment to take account of the synergistic effect of albumin and H_2O_2 (or other ROS) in promoting corrosion of Ti6Al4V.

4 Conclusions

1. The presence of H_2O_2 promotes corrosion of Ti6Al4V by increasing both anodic and cathodic reactions, leading to increasing release of Ti, Al and V with increasing concentration of H_2O_2 in physiological saline.
2. The presence of albumin strongly inhibits the cathodic reaction and slightly inhibits the anodic reaction of Ti6Al4V in physiological saline resulting in a lower OCP. During

anodic polarisation, a net active peak is observed following addition of albumin owing to suppression of the cathodic reaction. The presence of albumin causes a small increase in Ti release from Ti6Al4V in long-term immersion tests, which is attributed to the decrease OCP brought about by the inhibited cathodic reaction, leading to dissolution of Ti6Al4V in the active region.

3. In the presence of both H_2O_2 and albumin, there is a very much higher rate of metal release from Ti6Al4V compared with that in physiological saline alone. This can be attributed to an increased rate of the anodic reaction caused by H_2O_2 complexation of Ti and some suppression of cathodic reaction by adsorption of albumin, leading to a OCP in the active region of Ti6Al4V and a correspondingly high corrosion rate.

4. The corrosion attack was found to be greater in the β-phase of Ti6Al4V.

5. Standard tests that currently use physiological or phosphate-buffered saline may underestimate the rate of corrosion of Ti in the body since they neglect the synergistic effects of albumin and reactive oxygen species such as H_2O_2 that are found in peri-implant tissue.

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Disclosures

The authors have no conflicts of interest to declare.

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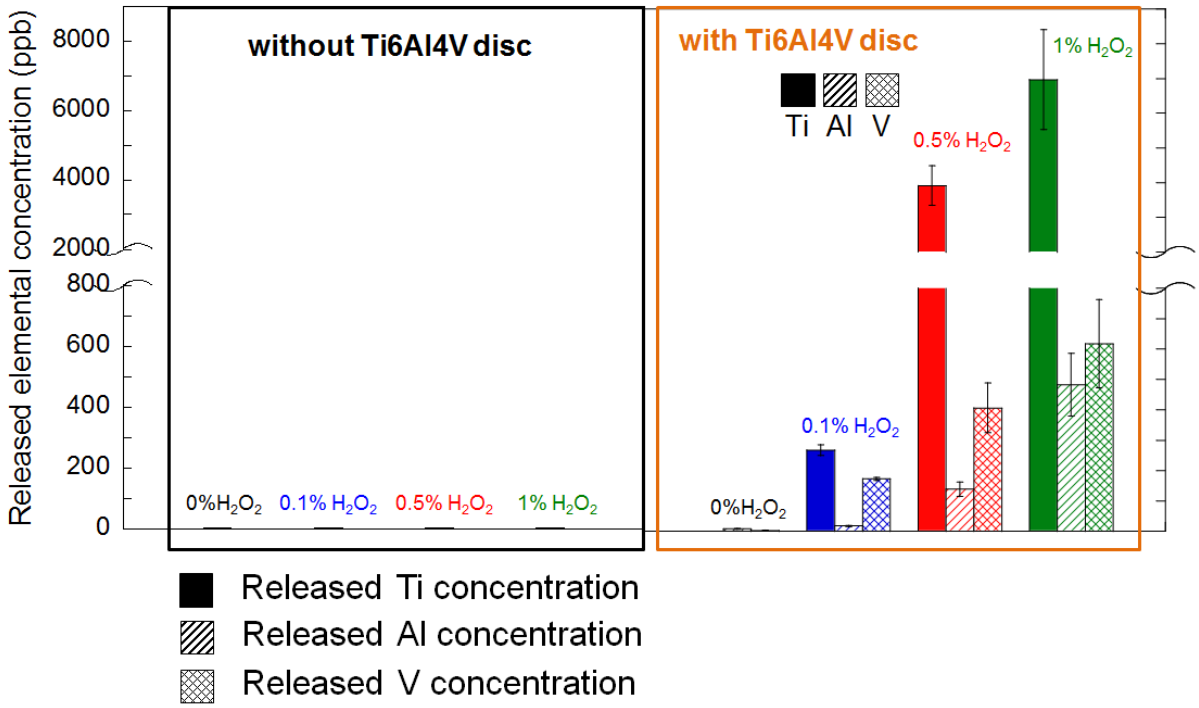


Figure 1 The mean concentrations (ppb) measured with ICP-MS of Ti, Al and V released from mirror-polished Ti6Al4V following immersion in physiological saline (0.15 M NaCl) with and without different levels of H_2O_2 for 4 weeks at 37 °C. Error bars refer to 1 standard deviation (n=3).

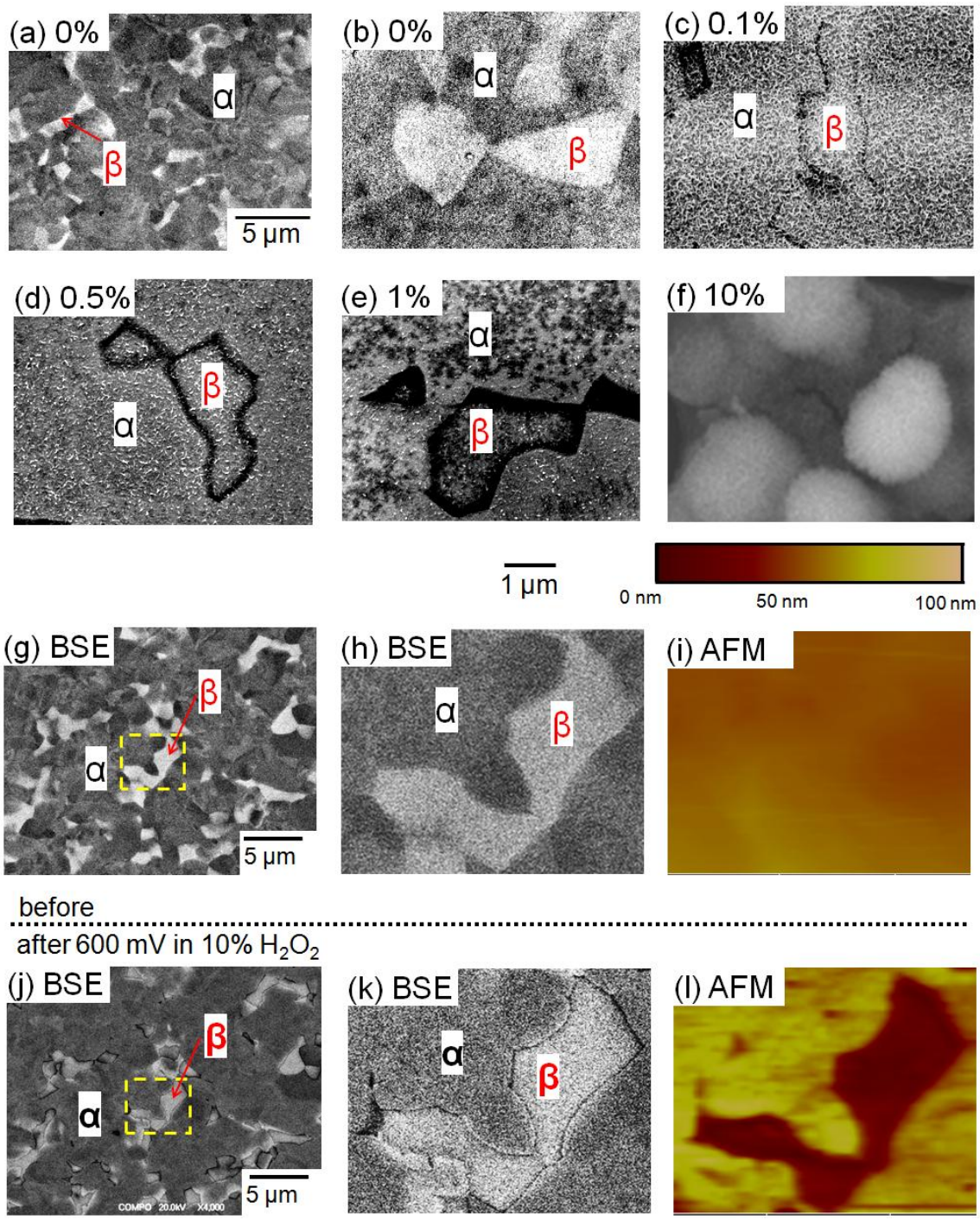


Figure 2 (a-f) surface morphologies of mirror-polished Ti6Al4V following immersion in physiological saline (0.15 M NaCl) with and without different levels of H_2O_2 for 4 weeks at 37 °C; surface morphology/topography of (g-i) before and (j-l) after potentiostatic measurement in 10% H_2O_2-containing physiological saline at 600 mV vs. SCE for 30 min at 37 °C. BSE: backscatter electron SEM image. Note that all micrographs are at the same scale except (a, g, j) which are at lower magnification as indicated. α phase and β phase were identified with EDX (data not shown) and BSE.

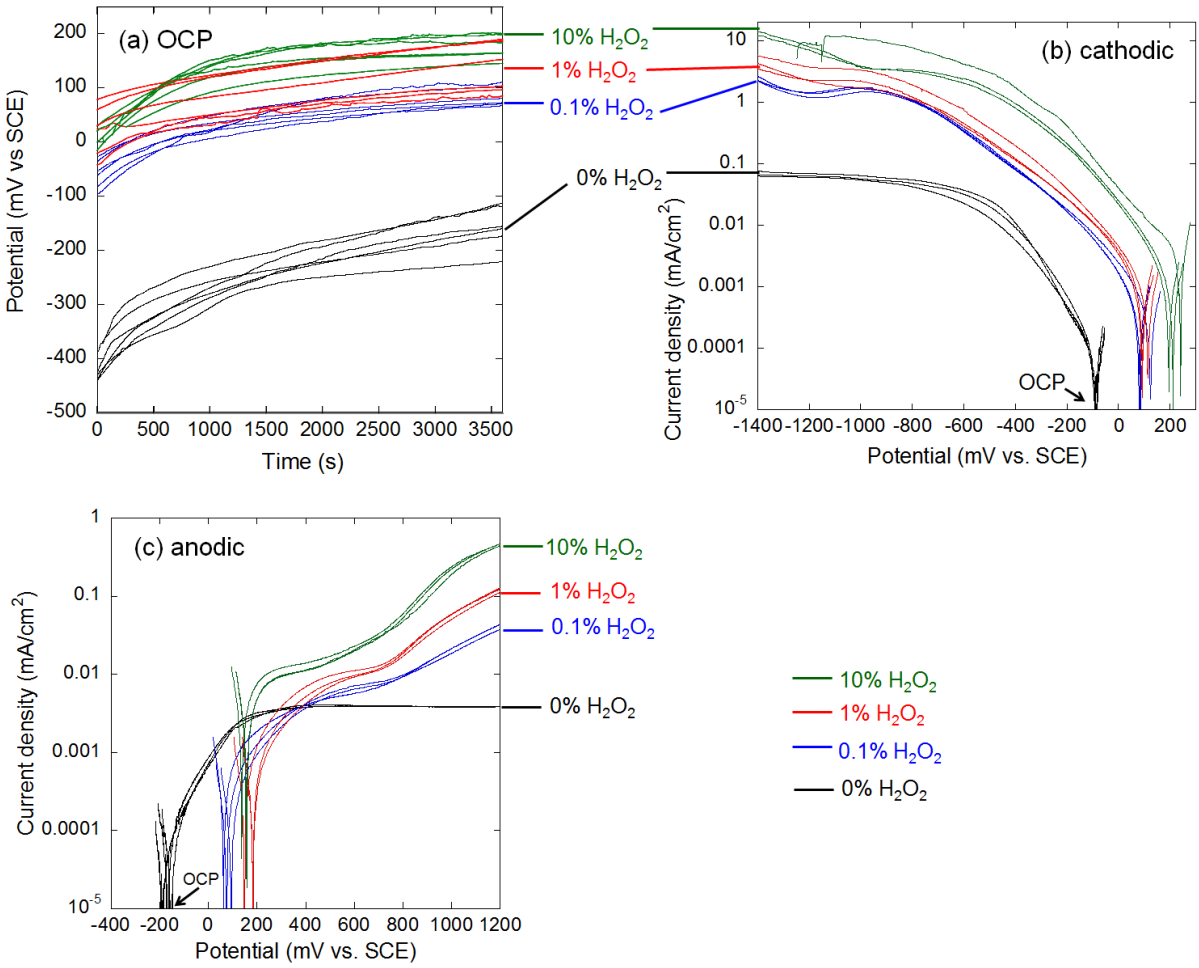


Figure 3 (a) OCP as a function of time; (b) cathodic and (c) anodic polarisation curves for mirror-polished Ti6Al4V in physiological saline (0.15 M NaCl) with and without different levels of H_2O_2 at 37 °C. The sweep rate was 1 mV/s.

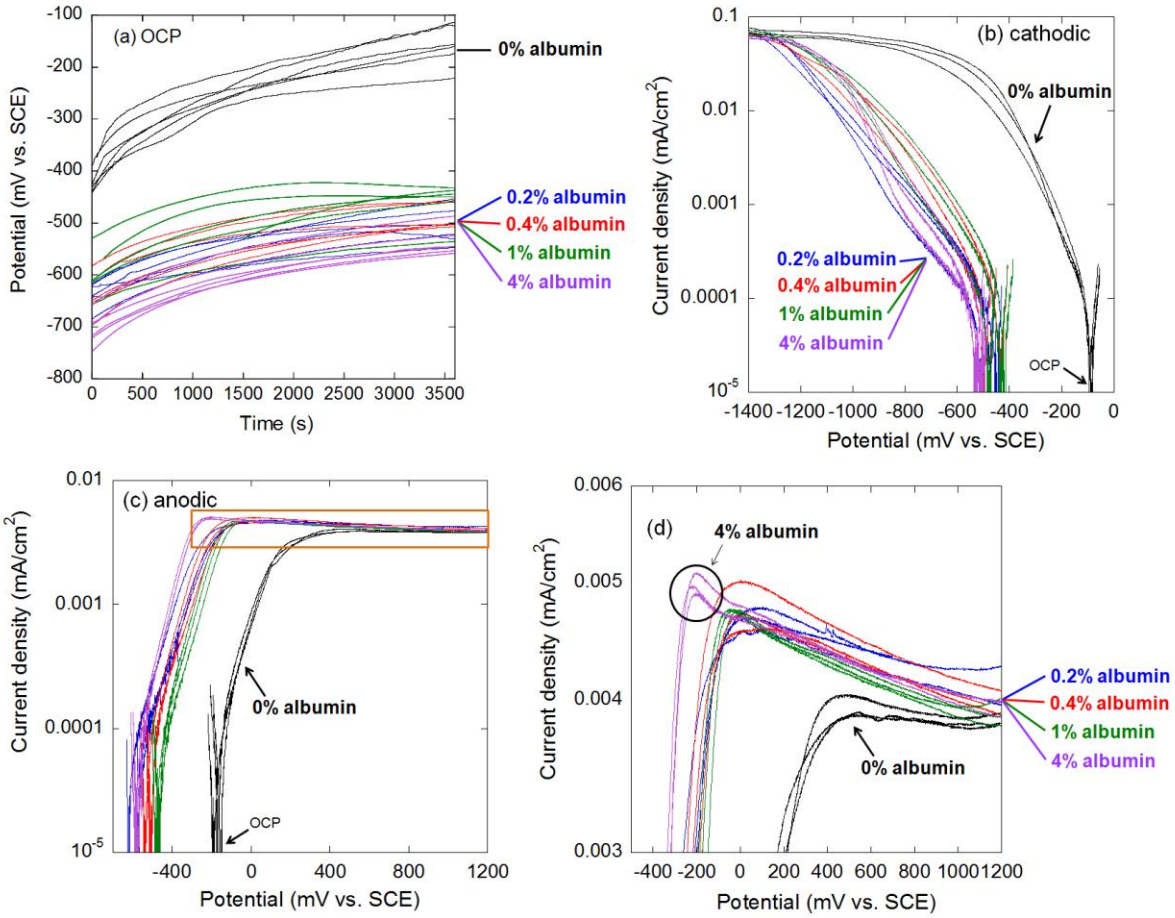
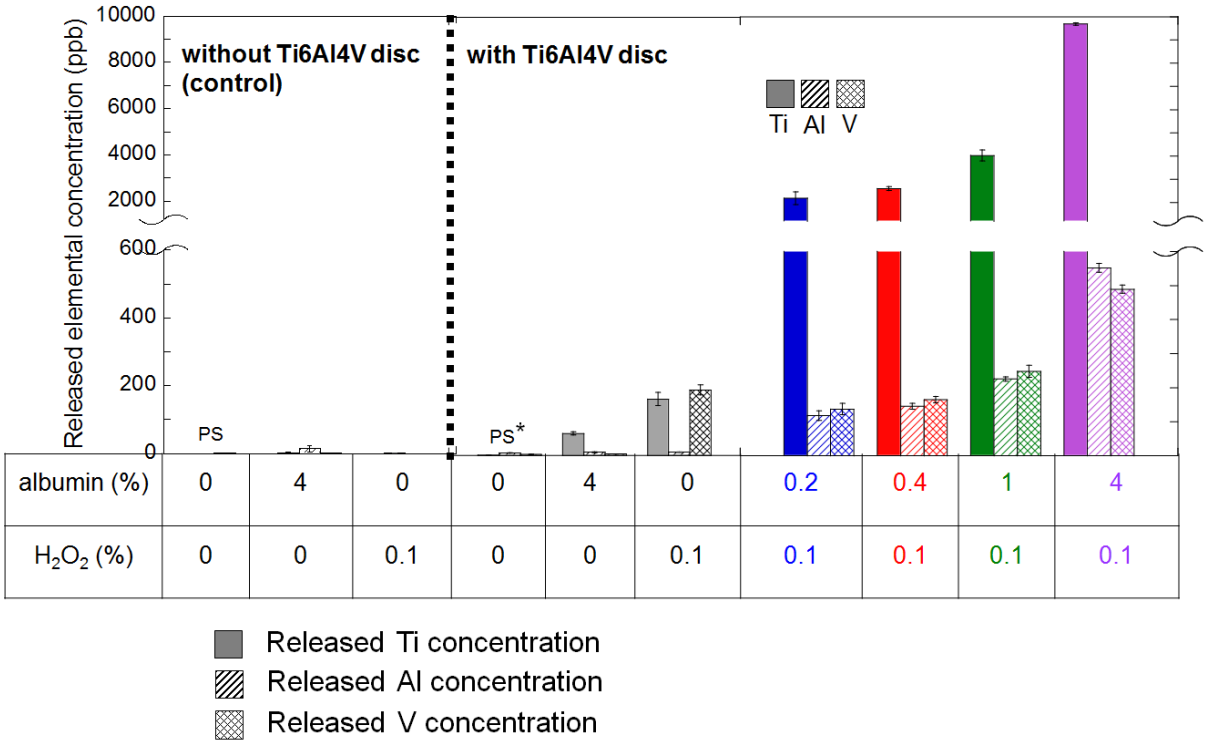


Figure 4 (a) OCP as a function of time; (b) cathodic and (c) anodic polarisation curves; (d) enlarged image of the region outlined in (c) for mirror-polished Ti6Al4V in physiological saline (0.15 M NaCl) in the presence and absence of different levels of albumin at 37 °C. The sweep rate was 1 mV/s.



albumin (%)	0	4	0	0	4	0	0.2	0.4	1	4
H_2O_2 (%)	0	0	0.1	0	0	0.1	0.1	0.1	0.1	0.1

Released Ti concentration
Released Al concentration
Released V concentration

Figure 5 The mean concentrations and standard deviations (ppb) measured with ICP-MS of Ti, Al and V released from mirror-polished Ti6Al4V following immersion in physiological saline (PS, 0.15 M NaCl) with and without H_2O_2 and albumin for 2 weeks at 37 °C (n=3); the results indicated * were from 4 week immersion test shown in Figure 1.

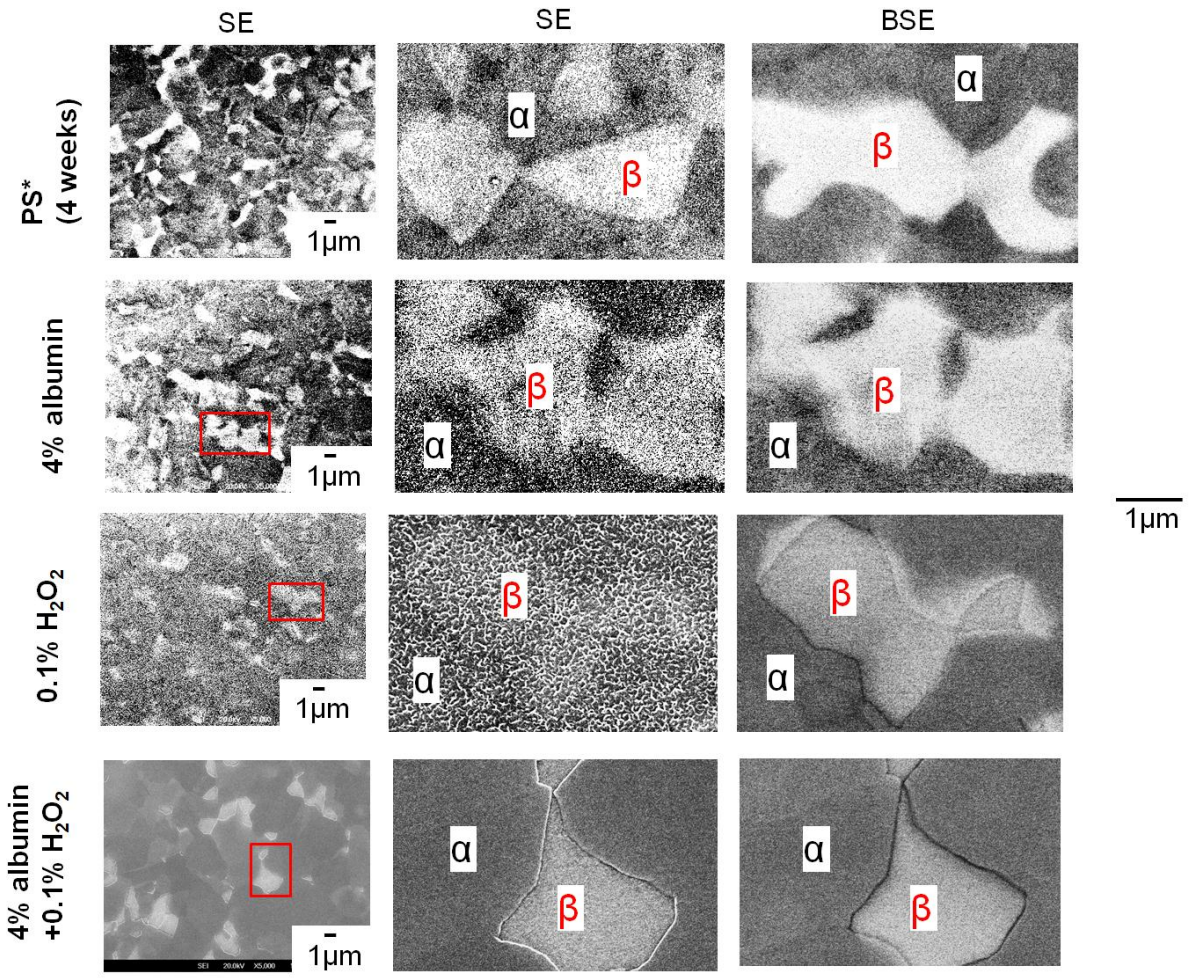


Figure 6 Surface morphology of mirror-polished Ti6Al4V following 2 week immersion in physiological saline (PS, 0.15 M NaCl) with or without H_2O_2 and/or albumin at 37 °C; the results indicated * were from the 4 week immersion test. SE: secondary electron SEM image; BSE: backscatter electron SEM image.

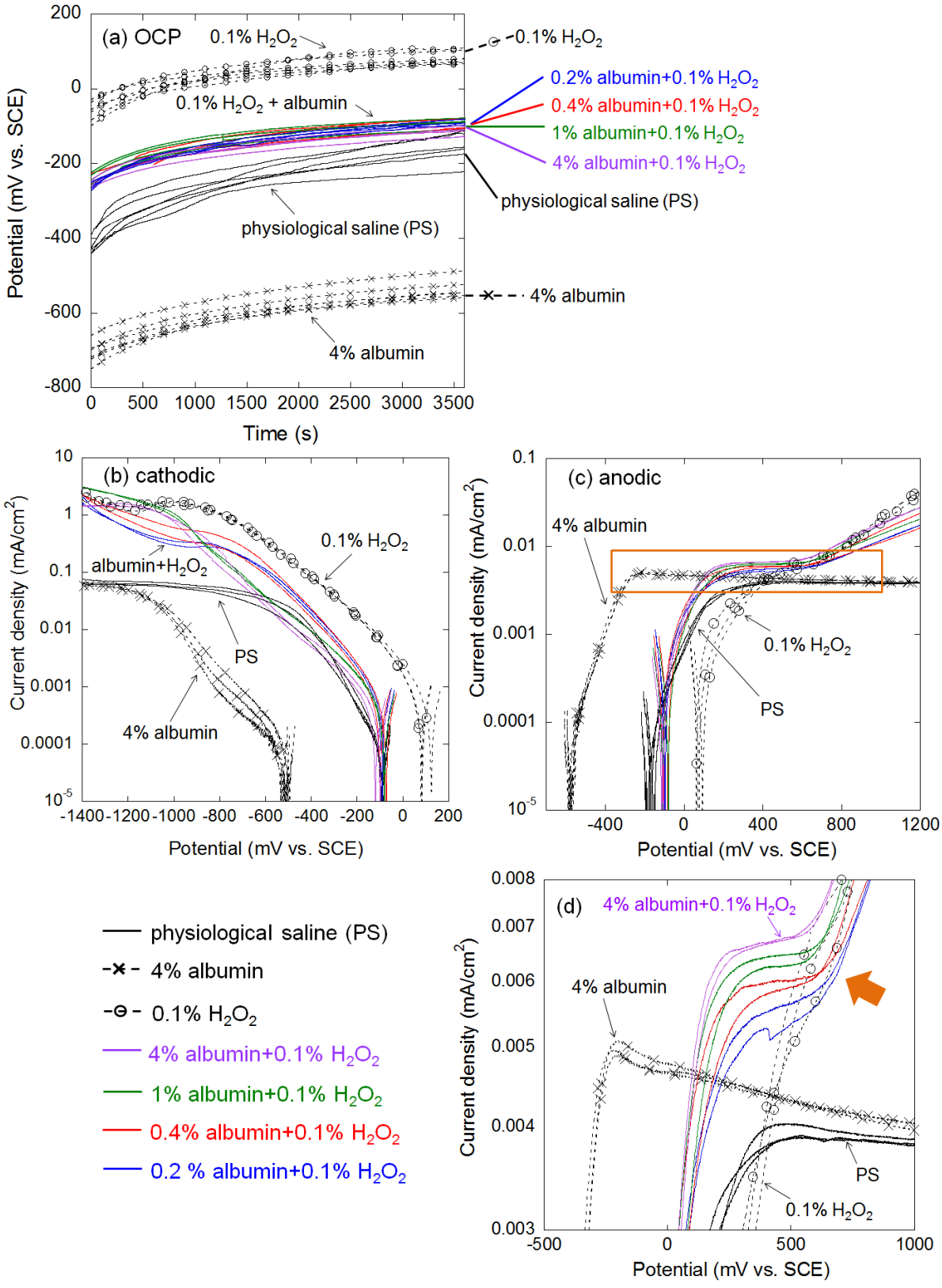
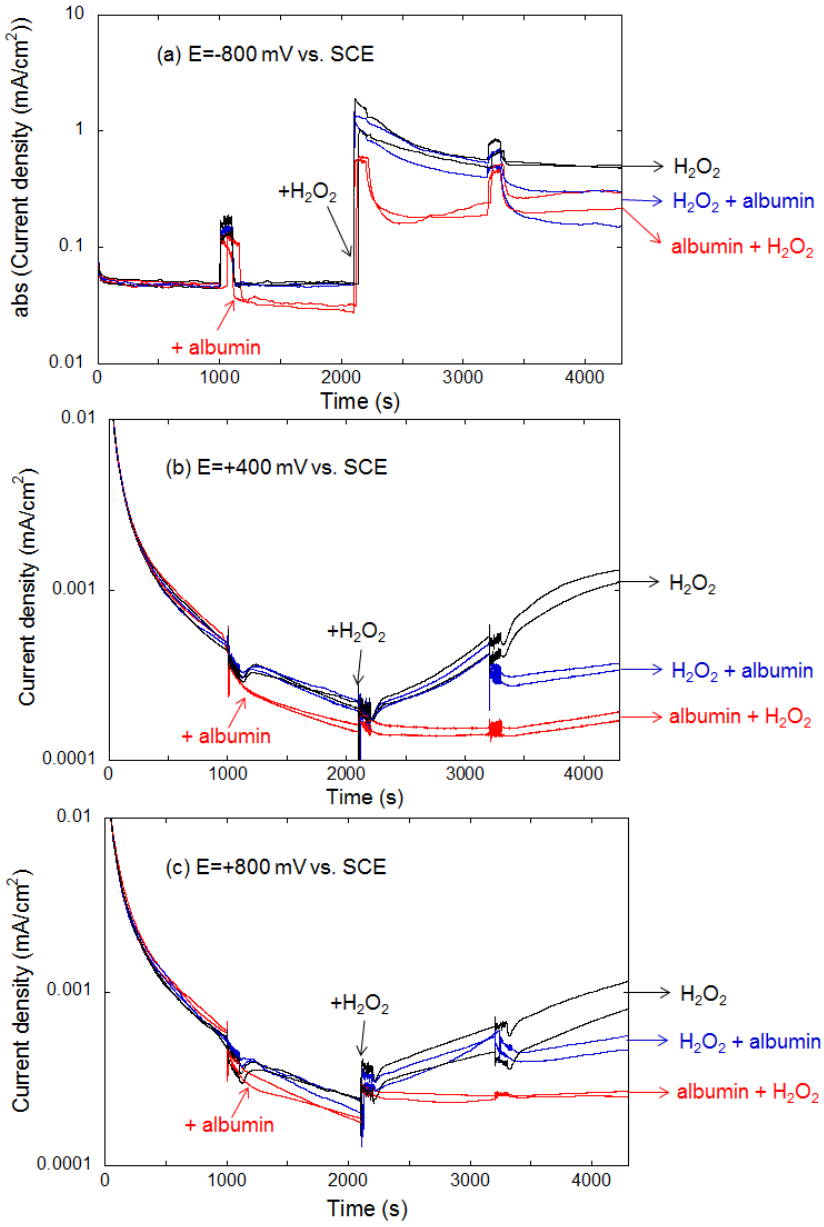


Figure 7 (a) OCP as a function of time; (b) cathodic and (c) anodic polarisation curves; (d) enlarged image of the region outlined in (c) for mirror-polished Ti6Al4V in physiological saline (PS, 0.15 M NaCl) with and without H_2O_2 and different levels of albumin at 37 °C. The sweep rate was 1 mV/s.



1st ~1000s	100s stir	2nd ~1000s	100s stir	3rd ~1000s	100s stir	1000s
PS	+1% albumin		+0.1%H_2O_2			
PS			+0.1%H_2O_2		+1% albumin	
PS			+0.1%H_2O_2			

Figure 8 Potentiostatic measurements of mirror-polished Ti6Al4V at (a) -800 mV; (b) 400 mV and (c) 800 mV vs. SCE in physiological saline (PS, 0.15 M NaCl) with addition of H_2O_2 and/or albumin at 37°C. The solution was stirred for ~100 s at ~1000 s intervals to ensure complete mixing; for some experiments, a specific amount of H_2O_2 or albumin (in PS) was added at ~1000 s intervals immediately before stirring to give a final concentration of 0.1% or 1%.

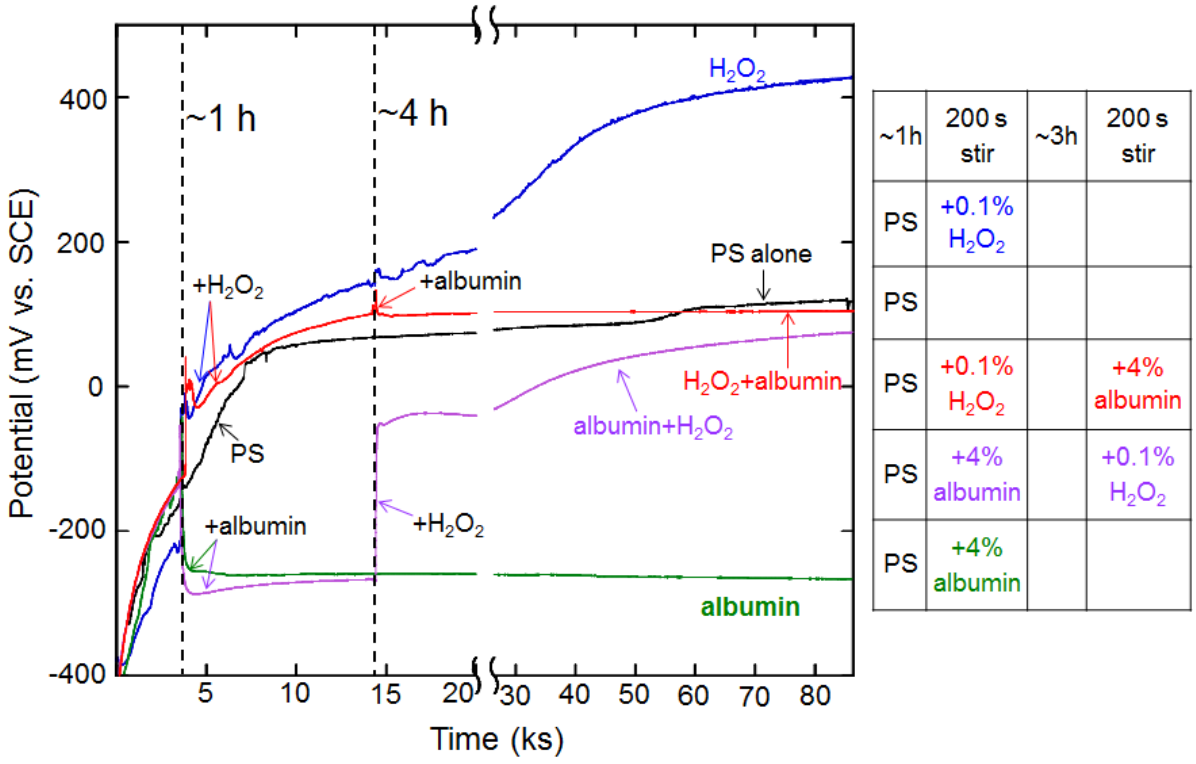


Figure 9 OCP as a function of time for 24 h for mirror-polished Ti6Al4V in physiological saline (PS, 0.15 M NaCl) with and without addition of H_2O_2 and/or albumin at 37 °C. The solution was stirred for ~200 s at ~1 h and ~4 h to ensure complete mixing; for some experiments, a specific amount of H_2O_2 or albumin (in PS) was added at ~1 h or ~4 h immediately before stirring to give a final concentration of 0.1% or 4%.

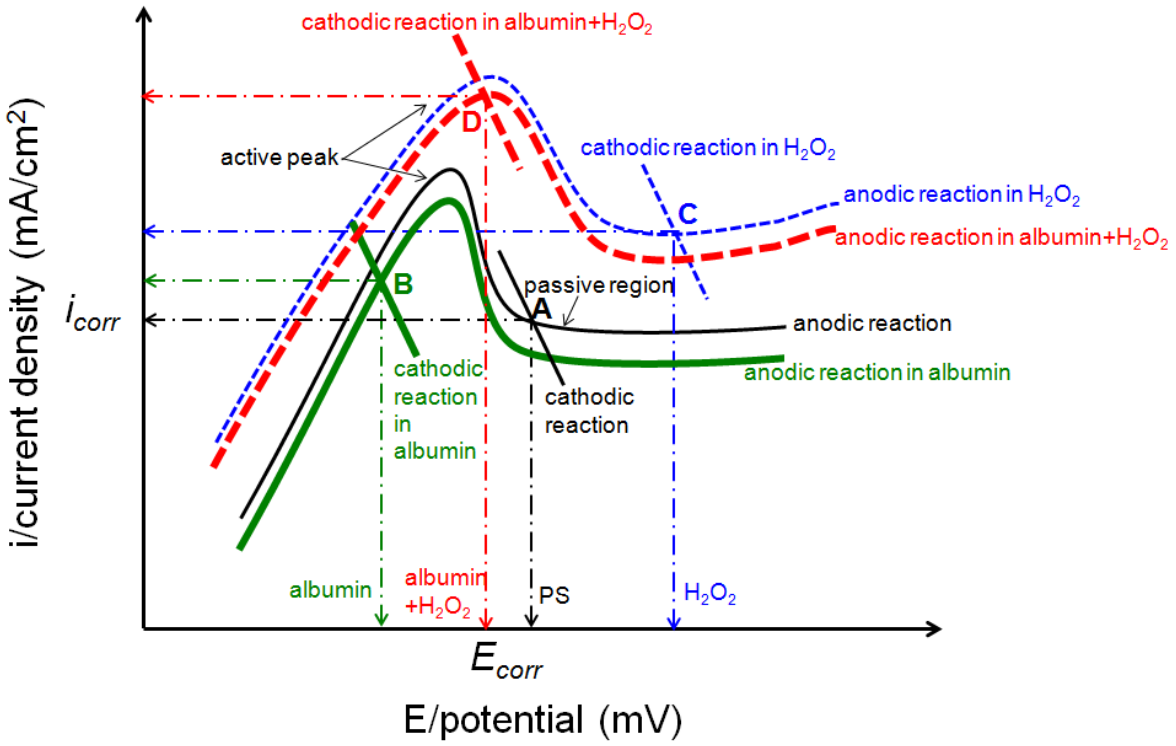


Figure 10 Schematic Evans diagram (mixed potential theory) to show the effect of H_2O_2, albumin and the combination of albumin and H_2O_2 on corrosion potential (E_{corr}) and corrosion current density (i_{corr}) of Ti6Al4V in physiological saline (PS).