Development and properties of Ti–In binary alloys as dental biomaterials

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The objective of this study is to investigate the effect of alloying element indium on the microstructure, mechanical properties, corrosion behavior and in vitro cytotoxicity of Ti–In binary alloys, with the addition of 1, 5, 10 and 15 at.% indium. The phase constitution was studied by optical microscopic observation and X-ray diffraction measurements. The mechanical properties were characterized by tension and microhardness tests. Potentiodynamic polarization measurements were employed to investigate the corrosion behavior in artificial saliva solutions with and without fluoride. In vitro cytotoxicity was conducted by using L929 and NIH 3T3 mouse fibroblast cell lines, with commercially pure Ti (CP–Ti, ASTM grade 2) as negative control. All of the binary Ti–In alloys investigated in this work were found to have higher strength and microhardness than CP–Ti. Electrochemical results showed that Ti–In alloys exhibited the same order of magnitude of passivation current densities with CP–Ti in artificial saliva solutions. With the presence of NaF, Ti–10In and Ti–15In showed transpassive behavior and lower current densities at high potentials. All experimental Ti–In alloys showed good cytocompatibility, at the same level as CP–Ti. The addition of indium to titanium was effective on increasing the strength and microhardness, without impairing its good corrosion resistance and cytocompatibility.

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1. Introduction

Titanium and titanium alloys have been widely used in biomedical devices due to their low density, excellent corrosion resistance and unique biocompatibility [1], among which ASTM grade 4 commercially pure Ti (CP–Ti) is the strongest unalloyed titanium and employed as dental prostheses material, possessing comparable tensile strength (550 MPa) to those of ADA type III dental casting gold alloys (530 MPa) [2]. However, it was found that the strength and hardness of CP–Ti were insufficient in some cases compared with conventional dental casting Co–Cr alloys [3,4]. Investigations revealed that the cast CP–Ti might be too soft and flexible for circumferential and bar retainers [5]. Therefore, dental restorations such as removable partial denture (RPD) frameworks must be designed well to prevent permanent deformation during installation or daily use [6]. This usually results in unwanted thickening of the prostheses, and uncomfortable feelings. Corrosion of titanium and titanium alloys in the oral environment is not a big problem except that in the presence of fluoride or H2O2. The attack by fluoride or hydrogen peroxide on the protective oxide on the surface can cause metal ion release and esthetic problems of discoloration [7,8], or even surface coarsening [9].

Aiming to improve the above mentioned deficiencies of CP–Ti, several binary titanium alloys, such as Ti–Cu [10], Ti–Ag [11,12], Ti–Mo [13,14], Ti–Cr [15], Ti–Au [16], Ti–Zr [17], Ti–Ta and Ti–Ir [18] alloy systems have been investigated experimentally. Each of the alloying elements has its own function. For instance, addition of Ag [11,12] and Cr [15] could increase the corrosion resistance of the alloys, especially against fluoride. Addition of Zr could not only increase the hardness and strength, but also reduce the melting temperature, thus facilitating the casting process [17].

There was also an attempt to add indium into titanium alloy [19], and surface analysis of alkali-heat treated Ti–In–Nb–Ta alloy indicated good bioactivity of the alloy [20]. Indeed, indium has been used for Ag-based and Pd-based porcelain-fused-to-metal (PFM) for a long time. The indium oxides film formed on the metal surface during the firing process of porcelain serves as a bonding agent between metal and porcelain [21,22]. Until now, no evidence of cytotoxicity of indium containing dental alloys has been reported, and in vitro cytotoxicity tests by Honez also confirmed the safety of indium [23]. Therefore, it is reasonable to employ indium as strengthening alloying element to improve the clinical performance of CP–Ti.

The purpose of this study is to investigate the addition of indium on the microstructure and mechanical properties of experimental Ti–In alloys, including Ti–11n, Ti–5In, Ti–10In and Ti–15In (in atomic percentage) alloys. Electrochemical techniques and in vitro cytotoxicity tests were also employed to assess the corrosion resistance and biocompatibility of Ti–In alloys.
2. Materials and methods

2.1. Sample preparation

Sponge titanium and bulk indium (99.95% and 99.995% in purity, from Beijing Mountain Technical Development Center for Non-Ferrous Metals) were used to fabricate the experimental alloys by arc-melting in argon atmosphere. The ingots of 30 g each were re-melt four times to ensure chemical homogeneity. The experimental samples were cut by electro-discharge machine directly from the as-cast ingots, with 1 mm × 2 mm × 60 mm in size for tensile tests and 10 mm × 10 mm × 1 mm for electrochemical and cytotoxicity tests. As reference specimens, titanium samples with the same size were cut from a sheet of commercially available grade 2 pure titanium.

2.2. Microstructure and mechanical tests

The square piece samples were mechanically polished with SiC papers and chemically etched with a solution containing 2–5 ml HF, 10–15 ml HNO₃ and 80–85 deionized water. Optical microstructure photograph of binary Ti–In alloys were obtained by using Zeiss Axiosvert 200 MAT microscope. Tensile tests of solution treated samples were conducted on an Instron 3365 universal testing machine with a strain rate of 0.01 min⁻¹ (3 samples for each test group). Before tensile testing, solution treatments were conducted at 1123 K for 1.8 ks to avoid composition segregation during cast procedure, and then the samples were water quenched to room temperature. Microhardness measurements were carried out on Shimadzu HMV microhardness tester, and five tests were conducted for each cast specimen. X-ray diffraction (XRD) for phase analysis was conducted by using an X’pert Pro diffractometer at 40 kV and 100 mA.

2.3. Electrochemical tests

A saturated calomel electrode (SCE) reference electrode and a platinum counter electrode were used for the corrosion test. A CHI 650C workstation was used to perform the open circuit potential and potentiodynamic tests. The test was maintained at 37 °C and the scan rate of potentiodynamic tests was 1 mV/s. The composition of electrochemical test solution was as follows: NaCl (0.4 g/L), KCl (0.4 g/L), CaCl₂ (0.78 g/L), Na₂HPO₄·H₂O (0.69 g/L), Na₂S·9H₂O (0.005 g/L), KSCN (0.3 g/L), and Urea (1 g/L). All the chemical reagents were of analytical purity. When NaF (2 g/L) was added, CaCl₂ was eliminated to avoid CaF₂ precipitation. The final pH of the solution was 5.2. After potentiodynamic tests, corroded surfaces of CP–Ti, Ti–5In and Ti–15In were examined by scanning electron microscope (Mx2600FE, Camscan, UK).

2.4. Cell viability tests

MTT colorimetric assay was employed to evaluate the in-vitro cytotoxicity of binary Ti–In alloys. In this method, the optical density of the culture solution, which is measured spectrophotometrically, is considered to represent the number of viable cells. Briefly, cellular enzymes could reduce MTT (3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide, a yellow tetrazole) to insoluble formazan, which gives a purple color. Therefore the number of viable cells could be obtained by measuring the color of the culture solution. Detailed description of this method could be found in [24]. Two mouse fibroblast cell lines L-929 and NIH 3T3 were adopted in this work. The cells were first cultured in Dulbecco’s modified Eagle’s medium (DMEM), with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5% CO₂ incubator. Extracts preparation were carried out in the incubator for 72 h with the surface area to DMEM volume ratio of 3:1. Extracts of grade 2 CP–Ti and DMSO were used as negative and positive controls, respectively. After 24 h incubating in 96-well cell culture plates, the medium was replaced with 100 μl extracts and incubated for 1, 2 and 4 days (n=5). After that, 10 μl MTT solution was added to each well, and then the samples were incubated for 4 h at 37 °C. Finally, 100 μl formazan solubilization solution (10% SDS in 0.01 M HCl) was added to each well. After 12 h, the spectrophotometrical absorbance of the samples was measured by microplate reader (Bio-RAD680) at 570 nm with a reference wavelength of 630 nm.

2.5. Ion release tests

Inductively coupled plasma atomic emission spectrometry (Leeman Profile ICP-AES) was employed to measure the concentrations of Ti and In ions dissolved into the cell culture medium after 72 h incubation. An average of three measurements was taken for each group.

2.6. Statistical analysis

The ion release content and cell viability data were analyzed statistically using one-way ANOVA method at the 95% confidence level (SPSS for Windows 16.0; SPSS, Chicago, IL).

3. Results

3.1. Microstructures and mechanical properties

Fig. 1(A) shows the representative optical micrograph of as-cast Ti–5In alloy, which was identical with the microstructure of other Ti–In alloys investigated in this study. A typical lamellar casting microstructure was found with clear grain boundaries, indicating a uniform single phase. The XRD patterns of as-cast Ti–In alloys shown in Fig. 1(B) confirmed that the single phase was hexagonal α-Ti phase. All the experimental Ti–In alloy samples were composed entirely of
hexagonal α-Ti phase without precipitates or second phase, which indicated that the alloying element indium below 15 at.% did not change the phase constitution of CP–Ti.

The tensile test results of solid solution treated CP–Ti and Ti-In alloys are showed in Fig. 2. The yielding strengths (YS) and ultimate tensile strengths (UTS) of Ti-In alloys were significantly higher (p<0.05) than that of CP–Ti, and the elongations were much higher as well, except for Ti–10In alloy. With the increase of indium, both YS and UTS of Ti-In alloys increased. Compared to CP–Ti (YS 262 MPa, UTS 393 MPa), YS increased to 414 MPa (by 58%), and UTS increased to 550 MPa (by 40%) for Ti–5In alloy; for Ti–15In alloy, YS increased to 539 MPa (by 105%), and UTS increased to 761 MPa (by 93%).

Fig. 3 presents Vickers hardness and bending modulus of CP–Ti and as-cast Ti–In alloys. As it is shown, the hardness of Ti–In alloys increased significantly with the increase of indium content. All of the Ti–In alloys exhibited higher micro-hardness than CP–Ti. In contrast, the bending modulus, all around 90 GPa, had nothing with indium content statistically.

3.2. Electrochemical corrosion behavior

Fig. 4(A) and (B) shows the variation of the open circuit potentials (OCP) of the experimental Ti–In alloys with immersion time in two solutions, artificial saliva and artificial saliva with 0.2% NaF addition. As it is shown, the addition of NaF yielded great difference on corrosion potential of the same Ti–In alloy. In the fluoride-free artificial saliva solution, the corrosion potential increased gradually at the early stage of immersion, and then varied slightly with immersion time after 1600 s. All the Ti–In alloys stabilized at more negative potentials than CP–Ti after 7200 s immersion. In the fluoride containing artificial saliva solution, the corrosion potentials decreased significantly in two steps to minima below —1 V before 2000 s. The first step was a progressive decrease of corrosion potential with time, indicating the progressive damage to the natural protective oxide of the experimental Ti–In alloys. The second step was a sudden drop of corrosion potential, indicating a sudden destruction of the original protective surface. Following this initial activation, the corrosion potential of the Ti–In alloys increased slightly for a while and stabilized after 7200 s immersion. It was noted that Ti–In alloys showed lower corrosion potentials than CP–Ti in fluoride-containing solution, which is consistent with that for the fluoride-free solution.

Fig. 4(C) and (D) shows the potentio-dynamic curves of CP–Ti and Ti–In alloys in fluoride-free and fluoride-containing saliva solutions. In the fluoride-free solution, both CP–Ti and Ti–In alloys showed similar polarization behavior. After cathodic polarization, all samples exhibited passivation behavior with almost constant passivation current densities regardless of the increasing potential. The passivation current densities of CP–Ti and Ti–In alloys were nearly identical, which were within the range of 7 and 10 μA/cm² at 2.5 V, indicating the same order of magnitude of corrosion resistance.

In the fluoride-containing solution, the experimental materials showed different passivation behavior compared with fluoride-free solution. They all exhibited clear active-passive transition peaks just above the corrosion potentials. Following this transition, CP–Ti, Ti–1In and Ti–5In alloys showed passivation behavior within a large potential range up to 2.5 V. Passivation current densities in this range varied from 100 to 372 μA/cm², two orders of magnitude higher than that in the fluoride-free solution. It is interesting to note that Ti–10In and Ti–15In alloys exhibited similar potentio-dynamic curves, which showed secondary active–passive transition peaks at around 0.5 V. Above this potential, the experimental alloys were passivated until 2.5 V, with passivation current densities of 87 μA/cm² and 92 μA/cm² for Ti–10In and Ti–15In alloys, respectively. The contrast of passivation behavior between the two solutions suggests the detrimental effect of F⁻ to the corrosion resistance of the Ti–In alloys.

Surface morphology of corroded CP–Ti and Ti–In alloys are showed in Fig. 5. As can be seen, CP–Ti, Ti–5In and Ti–15In alloys exhibited different surface characteristics. CP–Ti showed severely corroded morphology, exposing porous structure of substrate with oxide layer totally removed. The other two Ti–In alloys were much less corroded, with partial oxide layer left on the surface. Moreover, these two Ti–In alloys surface differed from each other. The substrate of Ti–5In alloy was also porous but with finer pores. This was just opposite to Ti–15In alloy, whose remaining oxide layer was porous and the substrate corroded uniformly. The different surface morphology of these two samples presented different corrosion mechanisms, probably due to the different content of indium and the oxides formed on the surface.

3.3. Ion release

Fig. 6 shows the Ti ion released into the cell culture medium (DMEM solution at 310 K) after 72 h incubation. It’s evident that there is little difference among all of the tested samples. The average concentrations of Ti were as follows: 10.00 ng/cm² for CP–Ti, 8.58 ng/cm² for Ti–1n alloy, 9.90 ng/cm² for Ti–5In alloy, 8.36 ng/cm² for Ti–10In alloy and 11.73 ng/cm² for Ti–15In alloy. Statistical analysis results showed that there was no significant difference between any two sample groups (p>0.05). The concentrations for indium ion were below the detection limitation, indicating very little amount of In ions released, partially due to the low content of In in the Ti–In alloys substrate.

3.4. Cell viability

Fig. 7 shows the viability of mouse L-929 and NIH3T3 fibroblasts, expressed as the absorbance ratio of the Ti–In alloy culture medium groups to that of CP–Ti (negative control) with error bars representing standard deviations, and DMSO was used as positive control. It can
be seen that, compared to CP–Ti group, the L-929 cell viabilities of all Ti–In alloy groups kept at around 100% after 1 day culturing. But they showed a little decrease after 2 days except for Ti–1In alloy group, and then the viabilities increased slightly for Ti–10In and Ti–15In alloy groups, in contrast to Ti–1In and Ti–5In alloy groups after 4 days culturing. The viability of positive control group decreased constantly with the culture duration, exhibiting significant cytotoxicity. One-way ANOVA results showed no significant difference between CP–Ti and Ti–In alloy groups (p>0.05) after 4 days culturing. The cell viability of Ti–15In alloy was significantly lower than that of CP–Ti group (p<0.05) after 4 d culturing, according to LSD multiple comparison results, but still above 90% compared with CP–Ti group.

In comparison with L-929, the viabilities of NIH3T3 for CP–Ti and Ti–In alloy groups show somewhat of difference. After 1 day culturing, the viabilities increased to above 110%, then decreased to around 80%, and then increased again to some extent equal to that after 1 day. The viabilities of the positive control group were all below 15%. Statistical analysis results suggest that the viabilities of Ti–In alloy extracts were significantly higher than that of CP–Ti extract after 4d (p<0.05, one-way ANOVA). Significant difference does not exist among Ti–In alloy groups.

4. Discussion

In order to provide a device or facility, certain specific property requirements pertaining to both the material and the product must be fulfilled. The material-specific properties include mechanical, physical, chemical and physicochemical properties, whereas biological properties such as biocompatibility are of additional importance. Intrinsic mechanical properties of metals and alloys are critically related to the microstructure, and can be tailored by means of mechanical processing, heat treatment and alloying. However, biocompatibility is generally related with the corrosion property of the material, since metal ions often release into the adjacent environment, during the corrosion process, and affect the tissues around it. Therefore, this study investigated the mechanical properties and cytotoxicity as well as the microstructure and corrosion behavior of experimental Ti–In alloys to examine their feasibility of using as dental alloys.

4.1. Microstructure and mechanical properties

According to the Ti–In phase diagram and Gulay’s work [25], when the content of indium increases to 33 at.%, Ti3In will precipitate in the as-cast Ti–In alloy. In present study, Ti–15In did not reach the α2 phase (Ti3In) region, as well as the fast cooling speed during the cast process, therefore no precipitate was found in the as-cast Ti–In alloys. As indicated by the optical micrograph and XRD patterns, all the Ti–In alloys consist of uniform hexagonal α phase, which is identical with CP–Ti, indicating that the addition of In does not change the phase constituent of CP–Ti.

Ohkubo pointed out that cast CP–Ti may be too soft and flexible for circumferential and bar retainers [5]. Tse and co-authors also found that the retentive force of CP–Ti clasps was approximately half that of Co–Cr clasps for the same undercut depth [26]. By employing simulation study, CP–Ti removable partial denture clasps showed lower retention force than identical Co–Cr clasps, over a simulated 5-year period [27]. Srimaneepong et al. investigated the deformation properties of removable partial dentures cast with Ti–6Al–7Nb alloy. It was found that Ti–6Al–7Nb alloy frameworks showed higher displacement and local strain than Co–Cr alloy frameworks, due to the lower elastic modulus of Ti–6Al–7Nb alloy [6]. According to the mechanical test results of
In alloys showed equivalent bending modulus with CP–Ti, indicating that the addition of indium would not lead to poor retentive force or deformation displacement (under the same load). Furthermore, due to the solid solution hardening effect of alloying indium, both strength and hardness were improved as shown in Fig. 2 and 3. The strength improvement was more effective than other alloying elements like Cu and Zr. By converting weight percentage to atomic percentage which was used in this work, YS and UTS of Ti–5Cu (corresponding 3.8 at.% Cu) increased by 45% and 13% [10], while the strength incremental ratio of Ti–5In were 58% and 40%. Accordingly, the bending strength of Ti–10wtZr (containing 5.6 at.% Zr) increased by about 22% [17], while the UTS of Ti–5In increased by 40%.

With the increased yield strength and the same elastic modulus, Ti–In alloys can be deformed to a larger strain without yielding. Therefore, this feature may increase the retention force by affording a larger elastic strain, and without permanent deformation or fracture for dental applications, such as removable partial denture frameworks, bridges and clasps. But it should be pointed out that the...
mechanical performances of a prosthodontics do not only depend on the mechanical properties, but also on the geometric design.

4.2. Corrosion and cytotoxicity

As is known, oxide layer on the surface of Ti provides protection and corrosion resistance. Corrosion occurs when the passive oxide film decomposed to soluble products, especially in the presence of fluoride [7–9,12]. It can be seen in Fig. 4, that the addition of NaF altered the electrochemical behavior of CP–Ti and Ti–In significantly, as shown in the decreased OCP and increased corrosion current density. This phenomenon was also found in pure titanium [28], Ti–6Al–4V [29] and Ti–Ag binary alloys [12]. The drop of OCP was considered to indicate a passive-to-active transition on the surface of alloys [12]. It is worth noting that both CP–Ti and Ti–In alloys showed delayed passive–active transition rather than immediate occurrence in the beginning in reference [28]. It is probably due to the difference of NaF content and pH between the present work and the literature.

Besides, Ti–10In and Ti–15In exhibited secondary active–passive transition as indicated in the potentiodynamic results. This situation demonstrates the “transpassive” dissolution of one oxide and leaving a stable one on the surface. The dissolution reaction under the corresponding potentials usually yields an increase of current [30]. With the increase of potential after that transition, these two alloys exhibited lower current densities. The surface morphology of the corroded samples (Fig. 5) suggested that both Ti–5In and Ti–15In had more protective oxides on the surface, which increased the corrosion resistance to some extent.

In vitro cell viability tests showed that Ti–In alloys have the comparable cytocompatibility with CP–Ti. The lowest cell viability was more than 90% for L-929 fibroblasts and 80% for NIH 3T3. Due to the limitation of this work, the decrease in NIH 3T3 viability on the second day is not well understood, further investigations are needed. However, after 4 days of culturing, the NIH 3T3 viabilities of all the Ti–In alloys were almost 110%, indicating good cell proliferation. Thus, the addition of indium did not alter the cytocompatibility of Ti. Investigation of metal cations cytotoxicity used in dental cast alloys showed that the TC50 (toxic concentration by which 50% of the cells in a culture are killed) of In+3 for L-929 fibroblasts was 2310 μM (265.23 mg/L) [31], which was much higher than that of other toxic metal ions such as Zn+2, Hg+2 and Cd+2, whereas, in the present study, the concentrations of In+3 released into the cell culture medium of Ti–In alloys were not detectable in ppb level, which were far below the above mentioned value. Ti ion concentrations were very low either, as determined by ICP-AES and compared with reference [8], in which Ti content released from CP-Ti was around 0.2 μg/cm² after 168 h immersion in H2O2 solution. All the Ti–In alloys had nearly identical Ti ion concentrations with CP–Ti. Brune reported that the daily dietary intake of Ti was approximately 20 cm², the released Ti ions from Ti–In alloys would be around 0.2 μg, which is insufficient to deteriorate the biologic safety of CP–Ti.

In this work, basic material properties of Ti–In alloys were evaluated primarily, with commercially pure Ti as reference. The binary alloys showed better mechanical strength, higher hardness, and equal in vitro cytotoxicity than CP–Ti. But further in vitro investigations such as casting procedure, wear resistance and porcelain compatibility are necessary to examine the potential of Ti–In as prosthodontic dental material.

5. Conclusions

The addition of indium to titanium from was found to be effective to increase the mechanical strength and microhardness without changing the phase constitution. Ti–In alloys exhibited similar passivation behavior with CP–Ti and the same order of magnitude of passivation current densities in artificial saliva solutions without fluoride. With the presence of NaF, Ti–10In and Ti–15In showed transpassive behavior and lower current densities at high potentials. In vitro cytotoxicity tests showed that Ti–In alloys had good cytocompatibility comparable to CP–Ti.

Acknowledgments

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