Candida albicans aspects of binary titanium alloys for biomedical applications

Shuyang Chen¹,², James K.H. Tsoi °¹,*, Peter C.S. Tsang³, Yeong-Joon Park⁴, Ho-Jun Song⁴ and Jukka P. Matinlinna¹

¹Dental Materials Science, Division of Applied Oral Sciences & Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Hong Kong SAR, People’s Republic of China; ²Department of Prosthodontics, Tianjin Stomatological Hospital, No. 75, Dagou Road, Heping District, Tianjin 300041, People’s Republic of China; ³Division of Restorative Dental Sciences, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Hong Kong SAR, People’s Republic of China; and ⁴Department of Dental Materials and MRC for Hard-tissue Biointerface, School of Dentistry, Chonnam National University, Gwangju 61186, Republic of Korea

*Correspondence address. Dental Materials Science, Division of Applied Oral Sciences & Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Hong Kong SAR, People’s Republic of China. Tel: +852-28-59-05-15; Fax: +852-25-48-94-64; E-mail: jkhtsoi@hku.hk

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Abstract

Titanium and its alloys are widely used in biomedical devices, e.g. implants, due to its biocompatibility and osseointegration ability. In fact, fungal (Candida spp.) infection has been identified as one of the key reasons causing the failure of the device that is inevitable and impactful to the society. Thus, this study evaluated the surface morphology, surface chemical composition and Candida albicans adhesion on specimens of 16 binary Ti-alloys (~5 wt% of any one of the alloy elements: Ag, Al, Au, Co, Cr, Cu, Fe, In, Mn, Mo, Nb, Pd, Pt, Sn, V and Zr) compared with cp-Ti, targeting to seek for the binary Ti-alloys which has the lowest C. albicans infection. Candida albicans cultures were grown on the specimens for 48 h, and colony forming units (CFUs) and real-time polymerase chain reaction (RT-PCR) were used to evaluate the biofilm formation ability. Scanning electron microscopy and confocal laser scanning microscopy confirmed the formation of C. albicans biofilm on all specimens’ surfaces, such that CFU results showed Ti-Mo, Ti-Zr, Ti-Al and Ti-V have less C. albicans formed on the surfaces than cp-Ti. RT-PCR showed Ti-Zr and Ti-Cu have significantly higher C. albicans DNA concentrations than Ti-Al and Ti-V (P < 0.05), whereas Ti-Cu has even showed a statistically higher concentration than Ti-Au, Ti-Co, Ti-In and Ti-Pt (P < 0.05). This study confirmed that Ti-Mo, Ti-Zr, Ti-Al and Ti-V have lower the occurrence of C. albicans which might be clinically advantageous for medical devices, but Ti-Cu should be used in caution.

Keywords: titanium; binary titanium alloy; C. albicans; fungal infection; medical devices

Introduction

Given the excellent mechanical properties and biocompatibility, titanium (Ti) and its alloys has become one of the most widely used materials for biomedical devices [1–4] as non-implantable (e.g. catheter [5], tweezer and scissors [6], dental partial dentures [7], dental bridges [8], bars and attachments [9] and orthodontic wires and appliances [10, 11]) and implantable devices (e.g. artificial joints [12], bone screw [13], fixation plate [14], pacemakers [15], scaffolds [16–18] and dental implants [4, 19–21]). Indeed, Ti can form a stable oxide layer, i.e. Ti oxides (TiO₅), as 3- to 10-nm thick film spontaneously with atmospheric oxygen and moisture. The stable ultrathin film could separate the bulk Ti material from its surrounding, and thus Ti has a high ability to resist the corrosion. Besides, the surface TiO₅ have shown to promote the exchange of calcium, cyclic nucleotides and inositol phosphates through the gap junction channels of osteoblasts [22–24]. So, this forms the basis of why Ti is able to bond with osteoblasts and is a good choice for intraosseous applications.
Even though Ti has shown numerous advantages, it also proceeded with some other drawbacks, e.g., low wear resistance, low deformability, and high reactivity with surrounding impurities, namely oxygen and nitrogen, at elevated temperatures [25, 26]. In particular to commercially pure Ti (cp-Ti), since it has a high melting point, it could not be processed easily by casting or additional manufacturing (a.k.a. 3D printing). The successful clinical case is rare [14]. Additionally, the bioactivity of TiOx on the Ti surface allows not only the useful cells such as osteoblasts and proteins, but also bacteria [27, 28] and yeast [29–33] to attach. For instance, Candida albicans is the major infectious yeast specie in medical devices such as catheters, joints, implant and dentures [34–36]. Study has shown in USA, C. albicans accounted for 15% of hospital-acquired sepsis cases, which are the fourth most frequent cause of blood stream infections largely due to medical devices infection [37]. The mortality rate due to this yeast-related infection in hospital was about 40% [38]. In fact, C. albicans is able to form biofilm alone on Ti surface, and even provide a hypoxic microenvironment which is a 'haven' to anaerobic bacteria, i.e. yeast and bacteria can positively to grow together [38]. Attempts such as drug coating [31] and silane coating [32] were tried to reduce the C. albicans on Ti with limited success, because the coatings can be dissolved under water. Furthermore, the drug coating would induce drug-resistance that might not be useful for long-term [20]. Therefore, yeast infection by medical device is a significant problem and is a hazard for human health and healthcare system.

To achieve (or improve) the mechanical properties with balancing a good biocompatibility and keeping low virulence of yeast, alloying Ti with a variety of elements might be a viable option. Ti-alloys are sensitive to their phases/crystal structures, and stabilisation of certain phases could be done by adding some alloying elements. The addition of the alloying elements can adjust the phase compositions (i.e., α, β and α+β) that change the bulk Ti-alloy properties. Thus, the mechanical properties of Ti might be enhanced and adjusted through alloying [39], such as increase the corrosion resistance, lower the modulus of elasticity and improve the machinability. Liu et al. has recently reviewed the mechanical properties, microstructure, chemical composition and processing of various binary Ti-alloys, and identified some metals can serve for the alloying elements, such as Al, Ag, V, Mn, Cr, Zr, Nb, Mo, Cu, In, Sn, Au, Pd and Pt [39]. Binary Ti-alloys have become a hotspot because two of the binary alloys have been commercialized: Roxolid® (Straumann, Basel, Switzerland), which is a dental implant based on Ti-Zr, and Ti-15Mo (Synthes, USA), which is used as orthopedic implant.

Furthermore, Park et al. [40] and Song et al. [41] evaluated the biocompatibility of various alloying elements in binary Ti-alloys. The studies revealed that the cytotoxicity of pure metals ranked in the order of: Al > Ag > V > Mn > Cr > Zr > Nb > Mo > cp-Ti [40] and Cu > In > Ag > Cr > Sn > Au > Pd > Pt > cp-Ti [41]. All the binary Ti-alloys from 5 to 20 wt% of alloy elements except Ti-10V have statistically similar biocompatibility with cp-Ti. Therefore, alloying with Ti might be beneficial to make a better material with superior mechanical and biological performance. The objective of this study was to test and evaluate the C. albicans aspects on binary Ti-alloys. The hypothesis was the types of Ti-alloys would have no significant effect on the C. albicans adhesion.

Materials and methods

Ti and alloys

Sixteen types of Ti-based alloys (with ~5 wt% of any one of the alloy elements: Ag, Al, Au, Co, Cr, Cu, Fe, In, Mn, Mo, Nb, Pd, Pt, Sn, V and Zr) and cp-Ti specimens were kindly supplied by Chonnam National University, South Korea, using a previously reported protocol [40, 41]. In brief, ~5 wt% of pure alloy metals were homogenized with Ti metal for 4 h at temperatures 150°C using vacuum arc melting technique under a high purity argon atmosphere on a water-cooled hearth. The mass was heated below to the respective alloy’s solidus temperature and then cooled to 600°C at a rate of 10°C/min before air-cooling to room temperature. The disc-shaped specimen were then cut into diameters in ~10 mm (Table 1) and measured by caliper. The disk surfaces were polished successively through 4000-grit SiC abrasive papers, and ultrasonically cleaned in acetone, ethanol and distilled water, before the following tests.

### Table 1. The wt% of alloying element and diameter for each Ti-alloy

| Sample | Alloying element (wt%) | Mean diameter (mm) |
|--------|------------------------|--------------------|
| Ti-5Ag | 5.04                   | 9.97               |
| Ti-5Al | 4.75                   | 9.97               |
| Ti-5Au | 5.26                   | 10.00              |
| Ti-5Co | 5.67                   | 9.98               |
| Ti-5Cr | 4.59                   | 9.99               |
| Ti-5Cu | 4.72                   | 9.64               |
| Ti-5Fe | 5.47                   | 9.93               |
| Ti-5In | 4.68                   | 9.95               |
| Ti-5Mn | 5.02                   | 9.95               |
| Ti-5Mo | 5.22                   | 9.60               |
| Ti-5Nb | 3.78                   | 10.00              |
| Ti-5Pd | 5.77                   | 9.95               |
| Ti-5Pt | 4.61                   | 9.92               |
| Ti-5Sn | 5.60                   | 9.90               |
| Ti-5V  | 4.37                   | 9.96               |
| Ti-5Zr | 4.89                   | 9.96               |
| cp-Ti  | N/A                    | 9.79               |

Sn, V and Zr] and cp-Ti specimens were kindly supplied by Chonnam National University, South Korea, using a previously reported protocol [40, 41]. In brief, ~5 wt% of pure alloy metals were homogenized with Ti metal for 4 h at temperatures 150°C using vacuum arc melting technique under a high purity argon atmosphere on a water-cooled hearth. The mass was heated below to the respective alloy’s solidus temperature and then cooled to 600°C at a rate of 10°C/min before air-cooling to room temperature. The disc-shaped specimen were then cut into diameters in ~10 mm (Table 1) and measured by caliper. The disk surfaces were polished successively through 4000-grit SiC abrasive papers, and ultrasonically cleaned in acetone, ethanol and distilled water, before the following tests.

Surface analyses

Scanning electron microscopy

To observe the morphology, one of the specimens from each group was prepared for scanning electron microscopy (SEM; Hitachi SU-1510 [VP-SEM, Tokyo, Japan]). They were fixed on aluminum stubs, and then observed with the acceleration voltage 15 kV in high-vacuum mode and the height of the electrode was ~15 mm. 500× and 2000× magnifications were used to overview and observe the initial condition of the specimen surfaces, and biofilm formation after the culture (Culturing of C. albicans and biofilm development section), respectively. Three different points from each specimen were chosen to record.

To observe the biofilm, specimens were fixed by immersing them for 1.5 h in 2.5% glutaraldehyde (BDH Lab. Supplies, UK). Subsequently, they were dehydrated by putting into a series of ethanol (70%, followed by 85%, 95% for once and absolute for twice) for 15 min. Finally, the dry specimens were sputtered-coated with Pd-Pt-coating.

Energy-dispersive X-ray spectroscopy

The chemical compositions of the 16 types of Ti-alloys and cp-Ti were analyzed with energy-dispersive X-ray spectroscopy (EDX) module (IXRF systems, Inc., Austin, TX, USA) that was mounted on the SEM. Three different spectra on the surface of the sample were analyzed with EDX. The silicon EDX detector was used, together
with the accelerating voltage 15.00 kV in high-vacuum mode. The magnification 500× was used and the area of observed spectra was ~500 × 500 μm². No element was excluded from the analysis and the number of iterations was six.

Microbiology
Sample allocation, pretreatment and post-treatment
For the microbiological tests, the cp-Ti was the control group, while the 16 types of Ti-alloys were the test groups. Before the culture, the Ti-alloys and cp-Ti were polished by 4000-grits SiC abrasive paper and pre-cleaned in an ultrasonic bath (Decon FS200; Decon Ultrasonics Ltd, Hove, UK) strictly following the cleaning protocol [(i) 95% ethanol for 5 min; (ii) Deionised (DI) water for 3 min; and (iii) acetone for 5 min] before each culture. This has been pretested [(i) 95% ethanol for 5 min; (ii) Deionised (DI) water for 3 min; and (iii) acetone for 5 min] before each culture. This has been pretested [(i) 95% ethanol for 5 min; (ii) Deionised (DI) water for 3 min; and (iii) acetone for 5 min] before each culture. This has been pretested. After the ultrasonic cleaning, the specimens were rinsed by the DI water and then steam autoclaved (Autoclave in-house that could completely remove the remnant biofilm and the solid residues were resuspended with 293 μl 50 mM ethylenediaminetetraacetic acid in eppendorf tube. A 15 μl lyticase (20 mg/ml) was added into each tube and they were incubated in a water bath (Jalabo TW12; Jalabo Laborteknik GmbH., Seelbach, Germany) at 37°C for 1 h. After that, the tubes were centrifuged at 13 000 rpm for 5 min. Again, the supernatant was discarded, but this time the spheroplasts were resuspended with the 180 μl tissue lysis buffer (Buffer ATL; QIAGEN GmbH, Hilden, Germany). A 20 μl proteinase K was added into each tube and vortexed, and the tubes were incubated in a water bath at 56°C for 10 min. Additionally, 200 μl lysis buffer (Buffer AL; QIAGEN GmbH., Hilden, Germany) was added to the samples and vortexed for 15 s. The tubes were then incubated in a water bath at 70°C for 10 min. A 200 μl absolute ethanol was then added and vortexed. The samples were applied carefully to the spin columns (QIAGEN GmbH., Hilden, Germany) without wetting the rim. The spin columns were centrifuged at 8000 rpm for 1 min and then replaced with a clean 2 ml collection tube. Five hundred microliters of wash buffer 1 (Buffer AW1; QIAGEN GmbH., Hilden, Germany) were added to the columns without wetting the rim. Then, the columns were centrifuged at 8000 rpm for 1 min and replaced with a clean 2 ml collection tube. Five hundred microliters wash buffer 2 (Buffer AW2; QIAGEN GmbH., Hilden, Germany) were added to the columns. These columns were centrifuged at 14 000 rpm for 3 min, again replaced with a clean 2 ml collection tube and centrifuged again at full speed for 1 min. Finally, the columns were transferred to a new 1.5 ml eppendorf tubes, with addition of 100 μl elution buffer (Buffer AE; QIAGEN GmbH., Hilden, Germany), then incubated at room temperature for 5 min with final centrifugation at 8000 rpm for 1 min. After these procedures, the DNA of C. albicans was extracted and collected, and then stored in the eppendorf tubes (‘cell solution’) at −21°C until the RT-PCR was carried out. On the other hand, the ‘master mix’ solution was prepared containing 5 μl of a nucleic acid stain (QuantFast SYBR Green; QIAGEN GmbH., Hilden, Germany), 2 μl DI water, 1 μl forward primer (5′ GGG TTT GCT TGA AAG ACG GTA 3′) and 1 μl reverse primer (5′ TGG AAG ATA TAC GTG GTG GAC GAC 3′).

To quantify the amount of C. albicans by RT-PCR, firstly standard curves by using known cell-concentrations solutions containing 10⁰, 10¹, 10², 10³, 10⁴ and 10⁵ cells of C. albicans have been generated by software (StepOne Software V2.2). Then, in separate wells of a 0.1 ml well plate (MicroAmp Fast optical 96-Wellplate; Applied Biosystems Pty Ltd., Scoresby, Australia), 1 μl of ‘cell solution’ was mixed with 9 μl ‘master mix’. The plate was covered with a cohesive cover (MicroAmp optical adhesive Film; Applied Biosystems Pty Ltd., Scoresby, Australia) and analyzed by a PCR machine (StepOnePlus; Applied Biosystems Pty Ltd., Scoresby, Australia). Each group was analyzed for six times.

Confocal laser scanning microscopy
The 48 h biofilm obtained from each specimen in Culturing of C. albicans and biofilm development section was washed with PBS,
and proceed with confocal laser scanning microscopy (CLSM). Then, they were stained by the LIVE/DEAD bacterial viability kit (Molecular Probes L7012) for half an hour and then observed under CSLM (IX81S1F-3, Olympus, Tokyo, Japan) using different magnifications. The pictures were analyzed by the equipment’s software (FV10-ASW 3.1 Viewer).

Statistical analysis

CFU and DNA concentrations (by RT-PCR) were statistically analyzed by using Kruskal–Wallis $H$ test and one-way ANOVA, respectively (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Results

Materials characterization

The morphological structure of the 16 types of binary Ti-alloys and cp-Ti was observed by SEM (Fig. 1) with 500× magnification. The polished Ti-alloys and cp-Ti surfaces are homogenous, slightly scratched with regularly distributed flaws in various size and shape. In particular, Ti-5Mn and cp-Ti have shown small microcracks (i.e. crevices).

Table 2 shows the EDX elemental analysis for the specimens after polished with SiC abrasive paper. In particular, Ti, C, O and the alloying elements have been evaluated. All the tested binary Ti-alloys well correspond to the information as per supplied (Table 1). Minute amount of Si and C was detected due to the use of SiC abrasive paper to polish the specimen in Ti-alloys and cp-Ti.

Microbiology

After the 48 h biofilm formation, SEM and CLSM (Fig. 2) were used to observe the attachment of the biofilm. Candida albicans could grow on all the surfaces. Figure 3 illustrated the median CFU/ml per unit area and the mean rank of the materials. When compared with the cp-Ti, with the alloy elements Mo, V, Al, Zr, Ag, Cr and Fe would give a lower prevalence of C. albicans adhesion in CFU/ml per unit area (mm²) on Ti-alloys. In general, the ascending order of C. albicans prevalence is:

- Ti-Mo < Ti-V < Ti-Al < Ti-Zr < Ti-Ag < Ti-Cr < Ti-Fe < cp-Ti < Ti-Pr < Ti-Nb < Ti-Co < Ti-Cu < Ti-Au < Ti-Sn < Ti-Pd < Ti-Mn < Ti-In.

However, Kruskal–Wallis $H$ test showed that there was no statistically significant difference in CFU/ml/mm² ($\chi^2 = 21.96, P = 0.144$) between groups.

RT-PCR results are shown in Fig. 4. PCR results revealed that only Ti-Cu and Ti-Zr groups have a higher C. albicans DNA concentration (per unit area) than cp-Ti. One-way ANOVA showed the DNA concentrations are highly statistically significant ($P < 0.0004 < 0.01$) between the groups, whereas Tukey HSD post hoc test Ti-Al and Ti-V have statistically lower DNA concentrations than Ti-Cu ($P < 0.05$) and Ti-Zr ($P < 0.05$). Ti-Cu also showed a high DNA concentration than Ti-Au, Ti-Co, Ti-In and Ti-Pr ($P < 0.05$).

Discussion

To the best of authors’ knowledge, this is the first study to compare the C. albicans biofilm formation ability on binary Ti-alloys. This study successfully cultured the biofilm on all the binary Ti-alloys.
and cp-Ti. Thus, it might also implies, if such Ti and alloys are used as medical devices, biofilm are possible to form on the surface. This study revealed that some alloying elements Mo, V, Al, Zr, Ag, Cr and Fe might give a lower CFU than cp-Ti, but not statistically significant. RT-PCR revealed that Ti-Cu seems to give the highest total C. albicans DNA concentration than others, and Ti-Zr surprisingly ranked the second, both are statistically higher than the lowest Ti-Al and Ti-V groups.

Fundamentally, RT-PCR and CFU are measuring different parameters and performance of the biological sample, which contains living (shown as green in Fig. 2 CLSM) or dead cells (shown as red in Fig. 2 CLSM). For CFU, the method is related to culture the C. albicans on agar plate, thus the measurement is related to those bacterium still alive. For RT-PCR, the method for detection is related to bind the DNA fragments of the cells with the primers, regardless the cells are living or dead. As a consequence, RT-PCR detects the total cell number.

 Furthermore, PCR utilized for the fluorescent emission technique which is very sensitive for the pH of the solution. As mentioned in Han et al. [20] and Liu et al. [4], in particular for Ti surface, at the atmospheric environment, small portion of Ti and Ti-OH at the surface would chemically react (by chemisorption) with the water moisture which might lead weakly bounded physisorbed water on the surface. In the condition of multivalent Ti (e.g. Ti$^{4+}$) metal, together with the physisorbed water that proceeds with the equilibrium dissociation reaction and becomes hydroxide (OH$^-$) and hydronium (H$^+$) ions, Ti is ready to form Ti-OH:

$$
\text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{OH}^- \quad (1)
$$

$$
\text{Ti}^{4+} + 4\text{OH}^- \leftrightarrow 4\text{Ti}^- + \text{OH}^- \quad (2)
$$

Then, the Ti-OH is likely to undergo further hydrolysis [43]:

$$
\text{Ti}^- + \text{OH}^- + \text{H}_2\text{O} \leftrightarrow [\text{Ti}-\text{O}]+ + \text{H}_3\text{O}^+ \quad (3)
$$

$$
\text{Ti}^- + \text{OH}^- + \text{H}_2\text{O} \leftrightarrow [\text{Ti}-\text{OH}_2]^+ + \text{OH}^- \quad (4)
$$

In theory, equilibrium reaction (3) would lead to the formation of basic type of TiO$_{x}$([Ti-O]$^-$) and the acidic type [Ti-OH$_2$]$^+$. These TiO$_x$ species are in nano thickness. Studies [44–46] had shown that the isoelectric point (IEP) for these TiO$_x$ at surface ranged from 5.0 to 6.7. The equilibrium reactions (3) and (4)

| Sample | Ti    | C    | O    | X    | Si   |
|--------|-------|------|------|------|------|
| Ti-5Ag | 91.327| 0.985| 0.000| 6.521| 1.167|
| Ti-5Al | 86.518| 0.882| 5.453| 6.116| 1.032|
| Ti-5Au | 87.173| 0.892| 6.469| 4.303| 1.163|
| Ti-5Co | 86.216| 0.576| 3.772| 7.756| 1.681|
| Ti-5Cr | 89.041| 1.306| 1.599| 6.331| 1.723|
| Ti-5Cu | 88.051| 0.773| 3.491| 5.587| 2.098|
| Ti-5Fe | 86.408| 0.581| 3.921| 7.783| 1.306|
| Ti-5In | 87.091| 0.986| 4.894| 6.066| 0.962|
| Ti-5Mn | 84.356| 0.151| 6.803| 7.015| 1.674|
| Ti-5Mo | 89.456| 0.277| 3.050| 5.950| 1.267|
| Ti-5Nb | 87.446| 0.000| 4.943| 6.427| 1.184|
| Ti-5Pd | 89.776| 2.620| 0.000| 6.595| 1.009|
| Ti-5Pt | 89.219| 0.415| 4.958| 4.489| 0.920|
| Ti-5Sn | 87.226| 0.338| 3.761| 7.333| 1.342|
| Ti-5V  | 86.853| 0.157| 6.337| 5.436| 1.216|
| Ti-5Zr | 87.747| 0.308| 5.854| 6.466| 0.900|
| cp-Ti  | 91.365| 0.404| 6.289| N/A  | 1.942|

X corresponds to the alloying elements.
suggested that, for environment pH is more acidic, i.e. lower pH, than IEP, the predominant oxide specie would be \([\text{Ti-OH}^2]^+\), and vice versa. Indeed, from Table 2, we knew that binary Ti-alloys (except Ti-Pd) and cp-Ti contains certain oxides. Thus, alloying Ti with various elements (and even to different alloying percentage [47]) would constitute different oxides and TiO\(_x\) content, i.e. various IEP. This said, the pH of the bacterium medium in Culturing of \(C.\) albicans and biofilm development section might be changed, such that the fluorescent emission of commercial PCR reagents might not correspond to the set standard curve (in Real-time polymerase chain reaction section). Therefore, to improve the RT-PCR experiment, the pH from each sample bacterium medium should be determined and standard curves in various pH should be drawn.

For Ti-Al and Ti-V, the biocompatibility was determined to be statistically comparable with cp-Ti [40]; however, the leaching of Al and V is inevitable [48]. Therefore, the release of these metal particles or ions could poison the \(C.\) albicans, because they possibly inhibit the formation of cell by interrupting the cell DNA synthesis. Hence, we could not find a high CFU values in Ti-Al and Ti-V, and the total \(C.\) albicans DNA concentration is less than others. In noble metals alloying elements, i.e. Ti-Ag, Ti-Pd, Ti-Pt and Ti-Au, they also have low DNA concentrations of \(C.\) albicans but a high CFU values, except for Ti-Ag. Pd, Pt and Au are bioinert that would not kill \(C.\) albicans. However, Ag has been demonstrated anti-bacterial effect due to its ‘zombie’ ability [49], whilst this anti-bacterial effect will need to happen in its cationic state. Thus, such a phenomenon might mean Ag from the alloy surface is releasing in Ag\(^+\) form, and the material mechanical properties might be affected. Further clarification is necessary.

Ti-Mo has been used as one of the commercialized orthopedic implant (Ti-15Mo, Synthes, USA), which performed the lowest CFU in this study. However, a careful selection about the percentage of Mo is necessary, since the existence of \(\omega\) phase [50] at low concentration of Mo (<15%) might have low temperature \(\omega \rightarrow \alpha\) transformation and thus affect the materials strength.

Ti-Zr, on the other hand, has been marketed as Roxolid\textsuperscript{®} dental implant (Straumann, Basel, Switzerland) in ~15% Zr. This study has shown Ti-Zr has the highest count on \(C.\) albicans concentration, but a relatively a low CFU count than cp-Ti and among others except for Al, V and Mo. Indeed this is an attractive Ti-alloy that posses with excellent biocompatibility and strength for biomedical applications. However, the processing is a challenge [51], i.e. for low concentration of Zr, the strength and elastic recovery (i.e. springback) properties for dental applications might not be sufficient due to the necessity of a harsh processing environment to reduce as much oxygen as possible. Nonetheless, the chemical similarity between Ti and Zr and acid etching ability would outcome all these environmental factors, such that Ti-Zr should pertain its foreseeable and fruitful future.

Ti-Cu has illustrated both high CFU and \(C.\) albicans DNA concentrations. Despite it might exhibit a good mechanical grindability and wear resistance [52, 53] that is good for CAD/CAM denture application [54], as well as good osteogenic and biocompatibility [55], the risk of \(Candida\) infectious contamination is high. Thus, cautious should be paid when using Ti-Cu as an implantable biomaterial in medical device.

**Conclusion**

Different types of binary Ti-alloys would not significantly affect the \(C.\) albicans adhesion is rejected. Binary Ti-alloys with 5 wt% of Mo, Al, V, Zr could reduce the occurrence of \(C.\) albicans which might be clinically advantageous for medical devices.
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Conflict of interest statement
None declared.

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