

Comparative Evaluation of Metal Ions Release from Titanium and Ti-6Al-7Nb into Bio-Fluids

Lori A. Joseph*, Omoniyi K. Israel**, Ekanem J. Edet*

ABSTRACT

Background: The study was designed to investigate the effects of pH, chloride ions and nature of some bio-fluids on the amount of metal ions released from titanium and TiAl₆Nb₇ plates following incubation in actual and simulated bio-fluids over time.

Methods: The amounts of released metal ions from commercially pure titanium (CpTi) and TiAl₆Nb₇ of surgical grade on immersion in 20 mL Hank's solution of pH 4.0 or 7.0, Hank's solution of high chloride ions concentration, Whole Blood Serum (WBS) and Phosphate Buffered Saline (PBS) at 37°C were determined over an incubation time of 20 weeks using atomic absorption spectrophotometry. The levels of released metal ions were compared by two-way ANOVA and Duncan's post-hoc tests. The amounts of titanium ions released by the samples were analyzed by Pearson's correlation.

Results: TiAl₆Nb₇ plate showed no release of Ti ions into the test solutions until after 12 weeks of incubation, while Ti ions were released from the CpTi plate from the 1 day immersion time. The release of measurable amount of Al ions from TiAl₆Nb₇ was after 12 weeks of incubation. The rate of release of Ti and Al ions from the samples increased initially with incubation time and then stabilized due to adsorption-desorption equilibrium.

Conclusion: The results showed that variations in pH and chloride ions of the test media has a significant effect on the amounts of Ti ions released, while increase in chloride ions concentration significantly elevates the release of Al ions into the bio-fluids.

Keywords: Biocompatibility, corrosion, ions, pH, titanium.

Received: September 2008

Accepted: December 2008

Dent Res J 2009; 6(1): 7-11

Introduction

Titanium (Ti) has been widely used in biomaterial fields.¹ Its high inertia due to the formation of a thin surface titanium oxide layer, light weight and excellent biocompatibility make it the ideal material for surgical implants.² The problems with poor mechanical strength of pure Ti were overcome by the addition of 6% aluminum and 4% vanadium to commercially pure Ti resulting in an alloy (TiAl₆V₄) with mechanical properties similar to those of stainless steel or Co-Cr alloys. Recent reports of the release of small amounts of aluminum (Al) and vanadium (V) into the human body - both being toxic to human body - has resulted to the Ti based alloy, (TiAl₆Nb₇) where V is substituted by niobium (Nb) and another (TiZr₁₅Nb₄Ta₄) where V is substituted by niobium (Nb) or tantalum (Ta)

and Al is substituted by zirconium (Zr). The pure Ti and Ti based alloys have inherent corrosion resistance attributed to the spontaneous formation of a strong passivating oxide layer. The extent of this corrosion resistance dictates their biocompatibility, useful lifetime and often their functional ability.³

When metallic implants are placed in the electrolytic environment provided by the human body, the electrochemical reaction resulting to the release of metal ions is coupled with a corresponding reduction reaction of constituents in the aqueous environment to maintain charge neutrality.⁴ How much of these metal ions are released is a function of the corrosion resistance of the metal, the physiological conditions (pH, chloride ion concentration, temperature, etc.), mechanical factors (pre-existing

*PhD of Chemistry, Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

**MSc in Chemistry, School of Basic and Remedial Studies, Ahmadu Bello University, Funtua, Nigeria.

Correspondence to: Omoniyi K. Israel, School of Basic and Remedial Studies, Ahmadu Bello University, P.M.B. 6007, Funtua, Nigeria. E-mail: israelflourish@yahoo.com

cracks, surface abrasion and film adhesion), electrochemical effects (galvanic effects, pitting or crevices) and the dense cell concentrations around implants.⁵

Metal ions generated by implants and their wear debris may remain in local tissues while some may bind to protein moieties that are then transported through the blood stream and lymphatics to remote organs. Although toxic effects of Ti, Al, V, Co, Cr and Ni in implant alloys have been reported, toxicology generally applies to soluble forms of these elements and may not apply to the chemical species that result from degradation of prosthetic implants.⁶

Ferguson et al were the first to document locally elevated titanium levels in the presence of a titanium implant.⁷ Relation between the fluoride concentrations and pH of corrosion environments on Ti ions leached from implants was investigated.⁸ Standardized artificial implant alloys (TiAl₆V₄, Ti, CoCr₂₉Mo and FeCrNiMoMnNbN) were immersed in inert polystyrene test tubes filled with 1 mL of serum (pH 7.4). The dissolution of the metal ions depended on the proportional mix of the individual elements in the respective alloys. The highest percentage of ion concentration was found for Co in CoCr₂₉Mo and the low percentages of ion concentration were evident for Ti (Ti), Cr (CoCr₂₉Mo) and Cr (FeCrNiMoMnNbN).⁹

This study aimed to investigate the effects of pH, chloride ions and nature of some bathing bio-fluids on the amount of metal ions released from Ti and TiAl₆Nb₇ plates following incubation in actual and simulated bio-fluids over time. A comparative assessment of the release of metal ions from Ti and its alloys is significant in elucidating the long-term biocompatibility profile resulting from alloying of the test materials.

Materials and Methods

Commercially pure titanium (CpTi) and TiAl₆Nb₇ plates (KS-50, Kobe Steel, Kobe, Japan) of surface area, 10mm x 10mm x 1 mm, were used as the test materials.

Bio-fluids for the study

1. Whole blood serum (WBS) was obtained by collecting fresh bovine blood of a healthy male cow into slantly positioned boiling tubes. WBS obtained after about 3 hours had a pH of 7.68.

2. Hank's solution (HS), to simulate body fluid –

was prepared with the following ionic composition (mol L⁻¹): Na⁺ 1.42 X 10⁻¹; K⁺ 5.81 X 10⁻³; Mg²⁺ 8.11 X 10⁻⁴; Ca²⁺ 1.26 X 10⁻³; Cl⁻ 1.45 X 10⁻¹; HPO₄²⁻ 7.78 X 10⁻⁴; SO₄²⁻ 8.11 X 10⁻⁴ and CO₃²⁻ 4.17 x 10⁻³.¹⁰

The solution was adjusted to pH 4.0 (HSpH4) and pH 7.0 (HSpH7) with 0.5M Na₂HPO₄ and 0.5M NaH₂PO₄ buffer solutions and drops of 1% HCl solution. The concentration of HS with high chloride ions (HSCl) was obtained by making the concentration of chloride ions in HS to be 1.63 X 10⁻¹ molL⁻¹.

3. Phosphate buffered saline (PBS) solution (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) with a pH of 7.45.

Invitro corrosion study

Corrosion was monitored by the amount of metal ions measured in the incubation biofluids. A plate of CpTi or TiAl₆Nb₇ was immersed in inert polypropylene bottle filled with 20 mL of Hank's solution, HS at pH 4.0, pH 7.0 or HS with high chloride ion (HSCl), the container was closed and incubated at 37°C. Three independent samples were prepared for each experimental group. Following incubation at the end of the 1 day, 1, 2, 4, 8, 12 and 20 weeks, 10 mL of each of the experimental solutions were withdrawn with a syringe after shaking. These were then digested with 3 mL of concentrated HNO₃ and 10 mL of concentrated HCl solution on a hot plate at 100°C. The digest was then made up to 20 mL with doubly distilled water.¹¹ The digestion was repeated for each of the experimental biofluids. These corrosion experiments were also performed for WBS and PBS.

The concentrations of Ti ions released from CpTi and also Ti and Al ions released from TiAl₆Nb₇ plates into the three mentioned bio-fluids were measured using a graphite furnace atomic absorption spectrophotometer (model TAS990, Intec Co. Ltd., Rome, Italy). The concentrations of these ions in the bio-fluids prior to incubation were taken as control. The quality assurance for the analyses was conducted through the spiking method, and mean percentages of recovery were calculated. The levels of released metal ions were compared by two-way ANOVA and Duncan's post-hoc tests (p < 0.05) and the amounts of titanium ions released by the samples were related by Pearson's correlation.

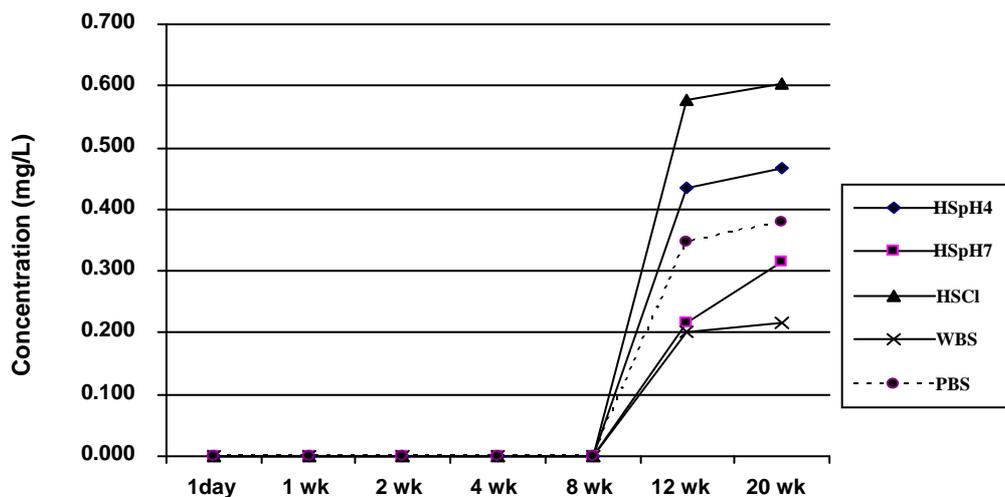


Figure 1. Titanium ions released from Ti-6Al-7Nb into bio-fluids

Results

The results are expressed as the average of triplicate determinations \pm the standard deviation and presented in Figure 1-2. Data was analyzed using statistical software (SAS), and a two-way ANOVA and Duncan's Post-hoc multiple range tests (DMRT) were used to assess differences in the mean values of the metal ions released due to effects of media and incubation time of the test materials in the bio-fluids. Pearson's correlation was used to find whether or not relationships exist be-

tween the amount of metal ions released by the two test materials at $p < 0.05$ levels of significance.

Discussion

Release of Ti ions

The results of this study showed that there was no detectable amount of released Ti ions from the $TiAl_6Nb_7$ samples into the various bio-fluids until after 12 weeks of incubation. The highest concentrations were measured in HSCl, with values 0.578 ± 0.001 mg/L after 8 weeks and 0.603 ± 0.002 mg/L after 12 weeks of incubation. The rate of

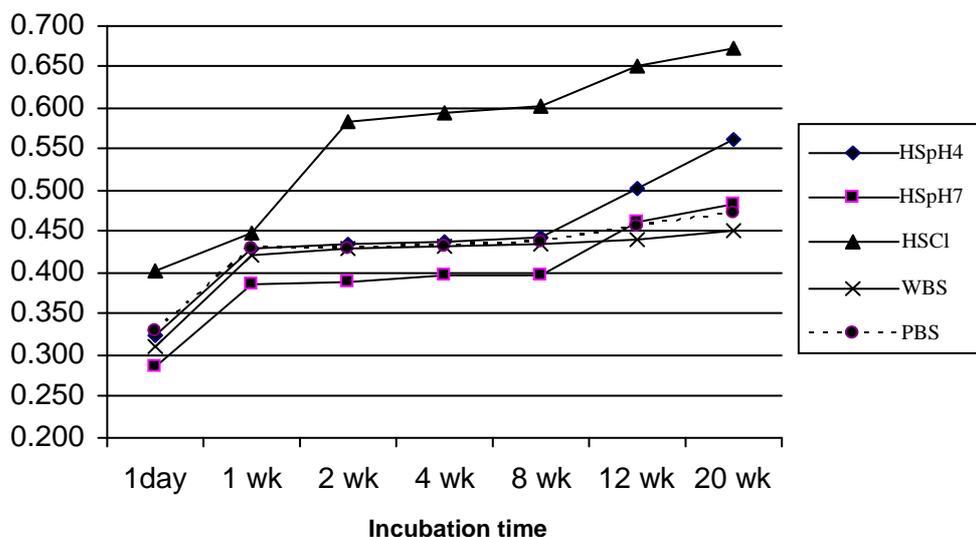


Figure 2. Titanium ions released from CpTi into bio-fluids

release of Ti ions from the Ti alloy ranged from 0.0167 ± 0.001 to 0.0503 ± 0.003 $\mu\text{g}/\text{mL}/\text{week}$ over the incubation time. The detection limit was $0.04\mu\text{g}/\text{mL}$ (Figure 1). DMRT showed a significant difference in the amount of Ti ions released into the various media, increase in chloride ions showed a strong difference and decrease in pH a less significant difference in the amounts of Ti ions compared to the other media. A statistically significant difference in the amount of Ti ions released from the sample was recorded beyond 8 weeks of incubation.

On the other hand, as shown in Figure 2, CpTi released measurable amount of Ti ions into the various bio-fluids from the 1 day incubation time. The chloride rich pseudo-body fluid (HSCl) and WBS resulted in significant release of higher mean concentration of Ti ions from CpTi compared to the other test solutions. There was no statistically significant difference between the amount of Ti ions released due to the effect of incubation time. The Ti ion release rate reaches a maximum after 1 week and then the rate of release diminishes with time. The rate of release of Ti ions from CpTi samples ranged from 0.023 ± 0.002 to 0.448 ± 0.004 $\mu\text{g}/\text{mL}/\text{week}$. The ranking from mild to aggressive corrosion media for the bio-fluids was HSpH7 < WBS < PBS < HSpH4 < HSCl. There was significant difference between the amounts of Ti ions released by the CpTi sample compared to that released by TiAl₆Nb₇. Pearson's correlation depicted a positive relationship in the mean

amount of Ti ions released into the bio-fluids by CpTi and TiAl₆Nb₇ ($r = 0.889$), with a highly statistical level of significance ($p < 0.05$).

Release of Al ions

The release of measurable amount of Al ions into the pseudo-body fluids were recorded after 8 weeks of incubation for HSCl and HSpH4 and after 12 weeks for PBS, WBS and HSpH7 as presented in Figure 3. The amounts of released Al ions into the five corrosion media after 20 weeks of incubation ranged from 0.060 ± 0.002 to 0.270 ± 0.004 mg/L . The detection limit was 0.02 $\mu\text{g}/\text{mL}$. DMRT showed a significant difference in the amount of Al ions released into the chloride rich environment (with release rate of 0.014 ± 0.001 $\mu\text{g}/\text{mL}/\text{week}$) compared to the other media, for instance, HSpH7 with Al ions release rate of 0.007 ± 0.001 $\mu\text{g}/\text{mL}/\text{week}$. Change in pH of the bio-fluid showed no statistical significance in the amounts of Al ions released, likewise extending the incubation time, until after 12 weeks. Pearson's correlation showed a positive relationship in the mean amount of Al and Ti ions released into the bio-fluids by TiAl₆Nb₇ ($r = 0.829$), but it was not significant.

This result conforms with that of Okazaki and Gotoh (2002) where the quantities of Ti ions released from Ti-15Zr-4Nb-4Ta, Ti-6Al-4V and Ti-6Al-7Nb alloys into phosphate buffered saline, α -medium and fetal bovine serum were very small compared to that in more acidic L-cysteine, 0.05 mass % HCl and 1 mass % lactic acid solutions.¹²

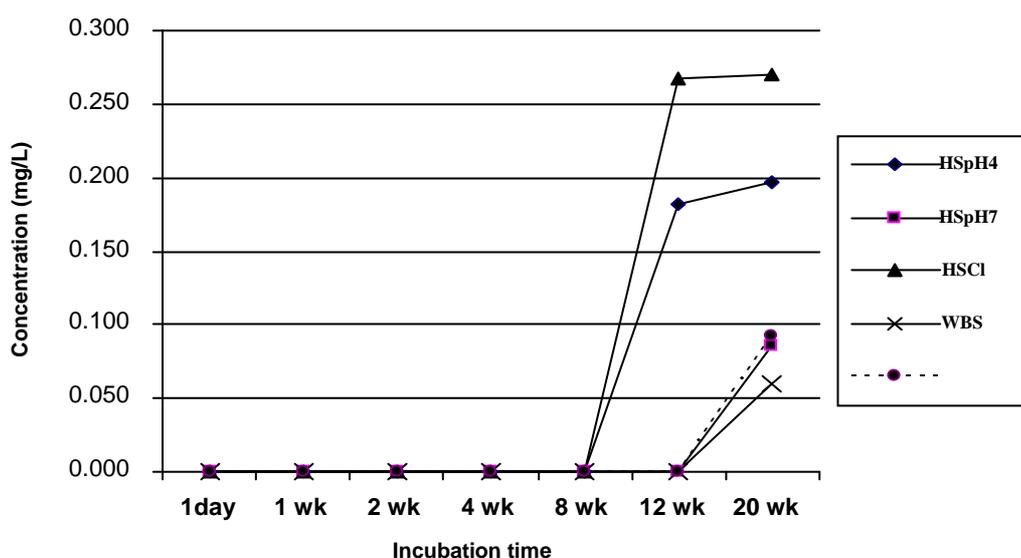


Figure 3. Aluminum ions released from Ti-6Al-7Nb into bio-fluids

Conclusion

In this study TiAl₆Nb₇ plate showed a remarkable resistance to the release of Ti ions into the test solutions until after 12 weeks of incubation compared to CpTi and this can be due to the presence of 6% Al and 7% Nb in the material. The rate of release of Ti ions from the Ti alloy ranged from 0.017 ± 0.001 to 0.050 ± 0.003 $\mu\text{g/mL/week}$. For CpTi plate of the same surface area, there was measurable amount of Ti ions released into the various bio-fluids from the 1 day immersion time. The rate of release of Ti ions from CpTi sample ranged from 0.023 ± 0.002 to 0.448 ± 0.004 $\mu\text{g/mL/week}$. There was a significant difference between the amounts of Ti ions released by the CpTi sample compared to that released by TiAl₆Nb₇.

The release of measurable amount of Al ions resulted after 12 weeks of incubation. HSCI had higher Al ions release rate (0.014 ± 0.001 $\mu\text{g/mL/week}$) compared to HSpH7 (0.007 ± 0.001 $\mu\text{g/mL/week}$). The results inferred that variations in pH and chloride ions of the test medium had a significant effect on the amounts of Ti ions released, while increase in chloride ions concentration significantly elevates the release of Al ions into the bio-fluids. The small amount of released metal ions measured in this study cannot constitute a health hazard to the host.

Notwithstanding, there is need for more long-term investigations of metal ions released from Ti and Ti based alloys into bio-fluids since patients hosts these biomaterials throughout life time, used either for dental and orthopedic replacements or as reported by studies for the systemic accumulation of metal ion in organs of post-operative patients of titanium implants and restorations.

Acknowledgement

The authors acknowledge the contributions of the Japan Society for the Promotion of Science and Prof. Takao Hanawa of the Tokyo Medical and Dental University, under the Asian-African Platform Programme through which funds to procure the metals and alloys were provided. Ahmadu Bello University Mac-Arthur Foundation is ac-

knowledged for provision of research to the second author.

References

1. Gronowicz G, McCarthy MB. Response of human osteoblasts to implant materials: integrin-mediated adhesion. *J Orthop Res* 1996; 14(6): 878-87.
2. McKinney RV, Lemons JE. *Dental Implant: Clinical and Biological Response of Oral Tissues*. 1st ed. St. Louis: Mosby. 1985. pp. 1-8.
3. Smith GK. Systemic aspects of metallic implant degradation. In: Rubin LR, Editor. *Biomaterials in reconstructive surgery*. St. Louis: Mosby; 1983. p. 229.
4. Litsky AS, Spector M. Biomaterials. In: Simon SR, editor. *orthopaedic basic science*. New York: American Academy of Orthopaedic Surgery; 1994. pp. 470-3.
5. Solar RJ, Pollack SR, Korostoff E. In vitro corrosion testing of titanium surgical implant alloys: an approach to understanding titanium release from implants. *J Biomed Mater Res* 1979; 13(2): 217-50.
6. Sunderman FW. A pilgrimage into the archives of nickel toxicology. *Ann Clin Lab Sci* 1989; 19(1): 1-16.
7. Ferguson AB, Jr., Laing PG, Hodge ES. The ionization of metal implants in living tissues. *J Bone Joint Surg Am* 1960; 42-A: 77-90.
8. Nakagawa M, Matsuya S, Shiraishi T, Ohta M. Effect of fluoride concentration and pH on corrosion behavior of titanium for dental use. *J Dent Res* 1999; 78(9): 1568-72.
9. Torsten M, Ralf M, Nikolaus J, Markus W, Wolfgang D, Rudolf M, et al. In vitro serum levels of metal ions released from orthopaedic implants. *European Journal of Orthopaedic Surgery & Traumatology* 2005; 15(2): 83-9.
10. Lori JA, Hanawa T. Characterization of adsorption of glycine on gold and titanium electrodes using electrochemical quartz crystal microbalance. *Corrosion Science* 2001; 43(11): 2111-20.
11. Van Loon JC. *Analytical Atomic Absorption Spectroscopy: Selected Methods*. 1st ed. London: Academic Pr; 1980. pp. 226-9.
12. Okazaki Y, Gotoh E. Implant Applications of Highly Corrosion-Resistant Ti-15Zr-4Nb-4Ta Alloy. *Mater Trans* 2002; 43(12): 2943-8.