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The Potential of Gamma Titanium Aluminides for Implant Applications

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Received: November 03, 2021; **Published:** February 26, 2022

Abstract

Gamma titanium aluminides, although originally developed for turbine applications at high temperature, appear to have the potential for biomedical implant applications. These materials, similar to Ti-6Al-4V, have excellent properties which may possibly extend their use to implants in the body. One advantage they offer over Ti-6Al-4V is the absence of vanadium toxicity. In this brief review, a wide gamut of experimental work is provided about second generation Ti-48Al-2Cr-2Nb (at.%) which has been interrogated for its biocompatible properties through a battery of experiments which include cytocompatibility tests with hFOB and MC3T3 cell lines and corrosion experiments in Ringer's solution with excellent results. Further improvement of the surface of this alloy by thermal oxidation indicates enhanced corrosion and wear resistance. Plasma electrolytic oxidation of the Ti-48Al-2Cr-2Nb surface demonstrated improved potential for osseointegration. In addition, both a rat model for alloy without surface modification and a sheep model with PEO treatment highlight the tremendous promise of gamma titanium aluminides for implant applications.

Keywords: *Gamma TiAl; Implants; Dental; Orthopedic; Osseointegration*

Introduction

Gamma titanium aluminides are titanium-based alloys which were originally developed with a strong emphasis on aerospace engine applications. These materials were initially investigated as a binary intermetallic (Ti-50Al)[1] in the early 1950s and appeared to possess good high temperature oxidation resistance [1,2]. Later in the 1980s and 1990s, a sizeable effort was dedicated to taking advantage of the excellent high temperature properties of gamma titanium aluminides to develop turbine blades [3,4]. A second-generation alloy, Ti-48Al-2Cr-2Nb was rigorously studied for engineering application after minor additions of Cr and Nb to the binary alloy to improve ductility and high temperature oxidation resistance over the long term [5,6]. Gamma titanium aluminide materials were further developed by minor changes in compositions, including small additions of Ta, Si, W and even Sn, in the same perspective as Ni- and Co-based superalloys. Based on different development efforts to improve properties while keeping application in turbine engines as the primary focus, gamma titanium aluminides now possess an excellent array of characteristics even though their ductility is lower than the other well-known titanium alloys [7]. In comparing the properties of gamma titanium aluminides with other titanium alloys Ti-6Al-4V (wt.%) appears foremost. Ti-6-4, has the advantage of higher ductility and hence offers the possibility of a variety of fabrication methods over gamma titanium aluminides, although the latter show the formation of a relatively more uniform and stable oxide layer and an ability to maintain strength and modulus at 700 °C – 800 °C. Gamma titanium aluminide with a density ranging from 3.7 – 3.8 gm/cm^3 has very high specific strength

[1]Composition of gamma titanium aluminide is conventionally expressed in atomic %.

and specific modulus which is attractive for flying turbine applications. Recent studies have highlighted the utilization of gamma titanium aluminides in potential dental and orthopedic implant applications [8-10].

Characteristics required for implant applications

Ti-6Al-4V has been used since the 1990s for orthopedic applications, initially as screws and pins but later in total hip and total knee arthroplasty. The same alloy has been also used successfully as dental implants. The characteristics that are critical for implant applications are mentioned below:

(a) **Strength**: For structural applications, strength is a basic requirement to withstand the stress fields experienced by the implant. Clearly, the implant design can be altered and fine-tuned to ensure that static strength requirements are met for functional success. Shear strength is also very important since many of the implants in real applications are subjected to bending loads and failure under this mode may be the most predominant. Furthermore, the fatigue properties of these materials in conjunction with implant design features become critical considering that titanium-based implants are expected to operate successfully for at least 20 years and used more profusely in younger patients taking their more robust lifestyle into consideration.

(b) **Rigidity**: This property is defined by the elastic modulus of the implant material, although it may be argued that in physical function the elastic nature of the implant as a whole may more pragmatically reflect its ability to bend and bear the applied load. It is important to mention that an important consequence of a material's elastic modulus is stress shielding, which is a phenomenon where the incompatibility between the elastic moduli of the tissue and the implant has been reported to lead to the lack of adequate load transfer to the tissue from the implant resulting in peri-implant tissue dysfunction and eventually implant loosening [11].

(c) **Wear resistance**: The implants are subjected to micromotion at a variety of interfaces, and hence wear properties of these titanium alloys also become important in maintaining the integrity of the implants in the long term [12,13]. Wear occurs primarily at implant-implant interfaces but also tissue-implant interfaces, although the latter is expected to be minimum in the overall scheme of implant design.

(d) **Corrosion resistance**: These metallic implant materials are expected to be in constant contact with synovial fluid and tissue and hence prone to corrosion. The pH of the body fluid is about 7.4 and hence titanium alloys regardless of the nature of their surface must be capable of resisting corrosion. Titanium alloys are normally subjected to pitting corrosion at the pH corresponding to body fluids [14-16].

While the above group represents the important material properties required for any application, in specific biomedical applications, other factors come into play which may eventually decide the suitability of the titanium material for the specific implant application. These are:

(e) **Cytocompatibility**: Implants with potential for application, when in contact with biological cells must ascertain that the cells survive and proliferate normally in the context of their physiological function. Early apoptosis or cell death in the peri-implant tissue is a deterrent to the adequate functionality of an implant. One critical aspect is the concept of integration of the tissue to the implant, called osseointegration in the specific case of hard tissue integration with the implant material [17]. The hydrophilic or hydrophobic nature of the implant surface, its chemical inertness, bioactivity (includes bioconductive and bioinductive characteristics) and its ability to conserve the genetic fidelity of nearby cells without untenable mutations are all key factors in determining the feasibility of using a certain material as an implant. Some materials have been reported to cause cytotoxicity and possibly genetic mutations resulting in implant rejection by the human body.

All these above factors have been lumped together in a term called biocompatibility with respect to actual implant usage. Unless this requisite of biocompatibility is stringently met by a potential biomaterial, the prospects for its use in implant applications is considered poor.

Testing for biocompatibility

A prospective biomaterial must undergo a series of qualifying tests before it can be considered for approval for use in implant applications. Other than the general materials testing protocols for static and dynamic strength properties, corrosion testing is imperative in a simulated body fluid environment. Literature reports testing media such as Hank's solution and Ringer's solution as viable alternatives [15]. Appropriate corrosion tests range from simple immersion and open circuit potential experiments to more sophisticated potentiodynamic polarization and electrochemical impedance spectroscopy methods. Wear testing of promising biomaterials is also very important, especially in applications where micromotion is a critical factor. It is recommended that wear and corrosion testing be performed simultaneously in simulated body fluid to understand the synergistic effects of both processes in the potential functionality of the implant material. It may be useful to carry out wear tests, specifically those involving reciprocating motion to simulate real applications. In fact, the use of hard tissue as one of the wear pairs may be specially revealing in terms of the characteristics of the biomaterial being tested.

Once the fundamental material characteristics of titanium alloys have met the required standards, the biological aspects in terms of compatibility has to be rigorously certified. The common experimental testing methods are:

(a) **Cytocompatibility**: These are experiments where the biomaterial under consideration is brought in contact with a specific line of cells. It makes sense that for replacement or structural materials in hard tissue applications, osteoblasts cell lines (hFOB, NIH 3T3, MC 3T3) should be natural choices. Primary cell lines would be ideal for testing but harvesting and cultivating these cells may trigger other issues associated with experimental protocols. Some groups have also used stem cell lines to study the effect of biomaterials surfaces on cell differentiation. Both, direct contact methods and elutant tests are valid for cytocompatibility. A number of commercial kits are available for testing cell viability while normal growth and proliferation of these cells on these biomaterials are also positive signs of cytocompatibility. Such assays are a common means of determining cytocompatibility but one important factor that many studies improperly ignore is the time period over which these cells are in contact with the biomaterial being tested. Many reports utilize a contact time of 24 hours to determine cell viability. Such a short test time period gives a haphazard result in that during this time of limited contact, many of the cells are barely beginning to establish contact with the substrate biomaterial. The cell configurations are yet to reach the typical morphology corresponding to appropriate cell contact with the biomaterial in this short period. At least 72 hours are required to estimate an acceptable degree of biocompatibility. Both, transformation of the biomaterial surface, as well as adequate time for cell interaction with the substrate will be enabled at relatively longer test durations for realistic observations. A longer exposure time of up to 21 days is ideal to not only arrive at conclusions about cell viability but also detect if the biomaterial allows for cell growth and proliferation. Some researchers have experimented with surface modification of biomaterials including compositional and morphological changes to study cytocompatibility. Oxidation and surface roughness are topological parameters which are important in cytocompatibility.

(b) **Animal models**: Once the prospective biomaterial has been approved with respect to cytocompatibility, further testing using animal models is the subsequent step prior to conducting clinical trials [16,17]. Depending on the envisaged use of the biomaterial in terms of physiological location, the appropriate animal model should be used. Animal models provide more relevant physiological information with respect to the effect of biomaterials compared to a cell line. In the case of animal models, the systemic response that is obtained is much more encompassing than the data obtained from cytocompatibility tests alone. The latter only provides the response to a potential biomaterial from one type of cell while much more information is garnered from the complex test environment offered by an animal model. Observation of the animal model over the appropriate implantation period will demonstrate the

real physiological effect of the implant material based on the normal physical behavior of the animal. Careful examination will reveal information about tumors, allergies and other functional and physiological deficiencies resulting from the presence of the implant in the animal model during the prolonged testing period. Programmed urine and blood serum analysis will elucidate the stability of the implant material in the tissue while imaging techniques such as X-ray, ultrasound etc. will throw light on the stability of the original position of the implant as well as its ability to integrate with the tissue.

A testing hierarchy is recommended in the use of animal models to investigate the biocompatibility of hard tissue implants based on the size of the animal model [18,19]. The smallest animals normally used for structural interrogation in bone tissue models include mice and rats [20]. The mouse and rat models are ideal for implantation of the test material either in the calvarial region or in the dorsal area. The implants are usually in the form of disks where the exposure of a large surface area of the biomaterial proximal to the tissue is purposed. Such contact tests involving intramembranous bone tissue provides information on the observation and results regarding biocompatibility based on toxicity of the implanted material or a dermal reaction to its presence. Experimental work involving implantation of small titanium or titanium alloy cylinders have been reported in studies involving the endochondral hard tissue of rats [21,22]. While these small animal models do provide reliable results on cytocompatibility, these tests are merely qualitative in terms of understanding the existence of adverse reactions to the presence of the biomaterial being tested considering the small size of the implants and therefore the small area of material exposed to tissue. Histological sections of the tissue from the area adjacent to the implant are examined to determine whether these biomaterials are deleterious to the peri-implant tissue based on cell morphology and function. Blood serum and urine samples from implanted rats can be analyzed to interrogate whether the biomaterial is stable or decomposing as a function of implantation time. Normally, acute, sub-chronic and chronic implantation periods are selected to study the degree of biocompatibility of the experimental biomaterial. Based on these initial results, the required detailed interrogation can be carried out with larger animal models. For quantitative data involving pull-out or push-out tests, larger animal models are chosen. The rabbit model is particularly useful since there is a balance between tissue size and manipulation of the animal during implantation and testing [23]. For implantation in hard tissue involving more invasive surgery, larger animals are more appropriate. Larger animals such as sheep or dogs have been used with an even greater degree of caution for implantation of biomaterials in hard tissue, although these are much more difficult to handle [24,25]. Recent trends in biomaterial testing have extensively leaned toward tissue and organoid models thus limiting animal use until the final stages of testing before eventual clinical application [26].

Evolution of gamma titanium aluminide as a potential biomaterial: testing and results

The promise of gamma titanium aluminide as an orthopedic and dental implant material has followed a logical testing sequence and has been clearly qualified at different stages to reveal its overall potential. The specific alloy used for this purpose was the second generation Ti-48Al-2Cr-2Nb which was received as hot isostatically pressed (at 1000 °C) 25 mm diameter rods which were about 30 cm in length. These alloys have the typical duplex microstructure consisting of 80% blocky gamma grains with 20% lamella composed of γ (TiAl) and α_2 (Ti_3Al). For most of the experiments, Ti-6Al-4V or commercially pure (cp-Ti) was used as comparison, while glass slides were used as control for studies involving cell culture. Repetitive tests were carried out to confirm results.

Cytocompatibility

Early testing of Ti-48Al-2Cr-2Nb consisted of seeding ATCC-11372 human fetal osteoblasts (1.19 hFOB) on polished disks (7 mm diameter and 1mm thickness) [27]. These disks were extracted from the as-received material and their surfaces were carefully prepared on both sides to obtain three different surface roughness: qualitatively, the disks were (a) ground to 600 grit, (b) mechanically polished using 3 µm diamond paste and (c) chemically polished with Mastermet solution. After the appropriate sterilization treatments, the disks were placed in 48-well culture plates. The human fetal osteoblasts were cultured on these disks at 37 °C and a 5% CO_2 atmosphere for a

duration of 21 days. Scanning electron micrography (SEM) revealed excellent growth of the human fetal osteoblasts on all three surfaces both on gamma titanium aluminide and Ti-6Al-4V. Cell growth appeared normal vis-à-vis glass slides with the appearance of lamellipodia and filipodia indicative of excellent cytocompatibility. To delve further, immunofluorescence labeling of two prominent proteins in osseous tissue was carried out to confirm the appropriate cell physiological functions. Both, collagen the most prominent structural protein, which was tagged using rhodamine and osteonectin, one of the bone sialoproteins and tagged using FTIC were observed qualitatively using laser scanning confocal microscopy after culturing the osteoblasts in surface-prepared disks. Gamma titanium aluminide appears to qualitatively demonstrate a higher expression of these proteins, although no significant differences were observed based on varying surface roughness. Figure 1 and Figure 2 depict the excellent cytocompatibility of gamma TiAl from observation through scanning electron microscopy and immunofluorescence microscopy.

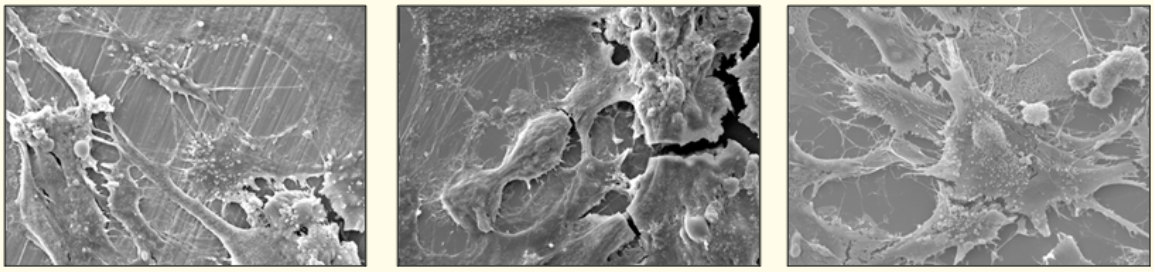

Figure 1

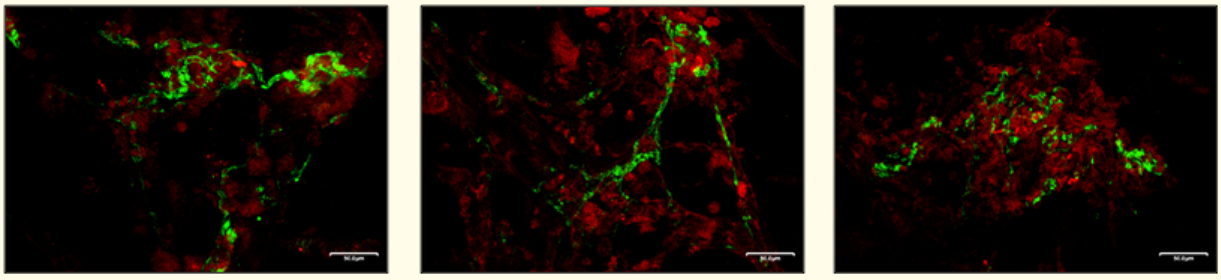

Figure 2

Figure 1 Scanning electron images of hFOB 1.19 cells cultured for 21 days at 37 ºC on γTiAl (L to R) with different surface roughness. Adapted from [27].

Figure 2 Immunofluorescence labeling of the expression of human collagen type I (Rhodamine labeled, red) and osteonectin (Cy5 labeled, green) in hFOB 1.19 cells cultured for 21 days at 37 ºC on γTiAl (L to R) with different surface roughness. Adapted from [27].

Corrosion testing

Ti-48Al-2Cr-2Nb disks were polished and subjected to potentiodynamic polarization testing in Ringer's solution using a K-cell [28]. Saturated calomel was used as the reference electrode and graphite rod was used as the counter electrode. In another set of experiments,

electrochemical impedance spectroscopy (EIS) was also used to evaluate gamma titanium aluminides. Table 1 provides a compendium of the EIS testing based on a valid fit of the data using an equivalent electrical circuit (EEC). These materials have very good corrosion resistance in Ringer's solution similar to Ti-6Al-4V, and although some degree of corrosion is observed, there is no concern with vanadium toxicity which is a real possibility during the corrosion of Ti-6Al-4V.

Surface Treatment	R_W ($W.cm^2$)	R_p ($MW.cm^2$)	C_{DL} (mF/cm^2)	R_{oxide} ($kW.cm^2$)	CPE_{oxide} (mF/cm^2)
Control	10.61	0.42	60.64	-	-
Autoclaved	8.53	9.02	11.6	-	-
Oxidized 500 C	27.3	23.92	2.12	-	-
Oxidized 800 C	14.12	7.93	57.24	1.04	2.45

Table 1: *AC impedance parameters for Ti–48Al–2Cr–2Nb (at.%) for all treatments [28].*

Surface modification of gamma titanium aluminide

In the initial stages of testing for biocompatibility, it was noticed that the Ti-48Al-2Cr-2Nb gamma titanium aluminides were prone to a minor degree of pitting corrosion despite the reputation of having excellent oxidation resistance. Preliminary corrosion testing indicated that although these alloys had a high resistance to uniform corrosion due to the formation of an oxide layer, pitting did occur in Ringer's solution [28]. To further increase the corrosion resistance of these gamma titanium aluminide alloys for implant applications, surface modification was attempted by thermally oxidizing the alloys at temperatures higher that 500 °C. Disks, 5 mm in diameter and 1 mm in thickness, were again extracted from the as-received material and metallographically prepared by grinding and polishing both sides of each disk. These were then oxidized in a furnace at 500 °C and 800 °C respectively. It is well known that these materials tend to form a mixed oxide of predominantly Al_2O_3 at 500 °C and TiO_2 at 800 °C. A bluish color corresponding to Al_2O_3 and a yellowish tint indicating TiO_2 are immediately visible after the oxidation process [28].

Cytocompatibility

As before, hFOB cells were brought in direct contact with the oxidized disks. In this case, Ti-6Al-4V disks oxidized at 500 °C and 800 °C were used for comparison [29]. As mentioned earlier, it is important to keep in mind the duration of contact of the cells with the biomaterial substrate. An interesting observation emerges from Figure 2. During the initial contact lasting 24 hours, the results indicate that all the surface conditions, autoclaved, oxidized at 500 °C and oxidized at 800 °C, give rise to the same level of cytocompatibility regardless of the titanium alloy. However, at longer duration of contact (7 days and 14 days) which is more of a reality in implant applications, it is very obvious from Figure C that the Ti-6Al-4V surface oxidized at 800 °C exhibits poor cytocompatibility. SEM observations results clearly show the presence of cell debris and a dearth of cells on this surface. As a caveat, if the 24 hours contact results were taken at face value, it would have been wrongly concluded that all surfaces mentioned above equally show very good compatibility. The absence of the expressions of the focal adhesion proteins vinculin and β1 integrin in parallel to the SEM data reveal the lack of cell adhesion on the surface of Ti-6Al-4V oxidized at 800 °C after 7 days. The toxicity of vanadium oxide VO_2 or V_2O_5 is most probably the cause of this phenomenon of cell death as reported in literature [30].

Figure 3 Hexosaminidase assay indicating cell viability after culturing human fetal obsteoblasts (hFOB 1.19) on gamma TiAl and Ti-6Al-4V substrates. GTi (control gamma TiAl, TiV (control Ti-6Al-4V), GTi5 (gamma TiAl oxidized at 500 C), TiV5 (Ti-6Al-4V oxidized at 500 C), GTi8 (gamma TiAl oxidized at 800 C), TiV8 (Ti-6Al-4V oxidized at 800 C) Adapted from [29].

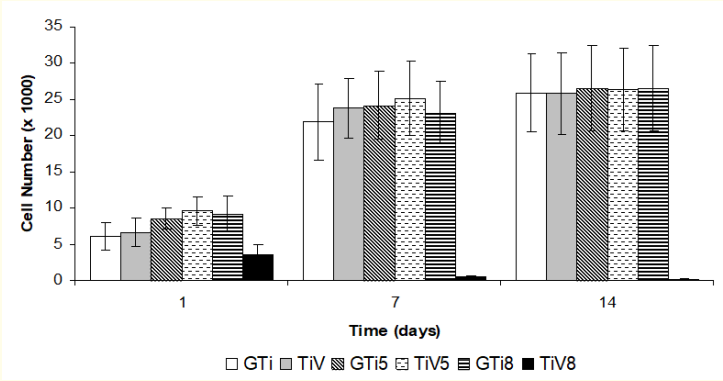

Figure 3

Wear testing

Disks of 25.4 mm diameter (1 mm thick) were obtained using EDM from Ti-48Al-2Cr-2Nb (at.%), Ti-6Al-4V (wt.%) and commercially pure (CP) titanium (95 grade). After griding to 600 grit, these disks were later oxidized in air at 500 °C and at 800 °C for one hour. A linear reciprocating tribometer was used to measure the wear resistance and friction of the oxide in each of the alloys in both dry environment (air) and lubricated environment (Ringer's solution) [31]. The wet and dry tests were executed with an applied force of 3N. The sliding velocity was set to 900mm/min at 5 Hz reciprocating tangential sliding amplitude. Also, the sliding distance was set to 10 mm. The pin used in these wear tests was obtained from bovine hard tissue which was appropriately shaped to make contact with the disk samples during the experiments. Results indicated that although abrasion was the dominant wear mechanism, the oxidized gamma titanium samples demonstrated lowest mass loss reflecting the high resistance of this surface to wear. Use of a lubricant during testing further decreased the mass loss lending credence to the excellent wear resistance of surface modified gamma titanium aluminides.

Attempts to increase the ability of gamma titanium aluminide for osseointegration

Although the tests of cytocompatibility and mechanical testing including wear testing are important for the overall biocompatibility of a material for implant applications, the critical importance of osseointegration with the biomaterial cannot be overstated. Various surface modification methods have been adopted for titanium alloys with a view to improve osseointegration [32]. These include simple techniques, both mechanical and chemical, to increase surface roughness, effective oxidation methods, increasing the hydrophilicity of the implant surface, augmenting bone morphogenetic and other proteins such as collagen, fibronectin, and growth factors to elicit prolific tissue increase on the implant surface. Of these, one very practical and popular method has been the plasma electrolytic oxidation (PEO) or microarc oxidation (MAO) process. Variants of this PEO method have been suggested as a means to surface modification to improve osseointegration [32].

Plasma electrolytic oxidation (PEO)

Plasma electrolytic oxidation is a promising surface modification process where the titanium-based biomaterial, gamma titanium aluminide was subjected to oxidation at relatively high voltages [33]. Although anodization of the metallic biomaterial occurs in the initial stages of the process, increasing the voltage beyond dielectric breakdown of the thin oxide layer results in a thick, highly adherent and porous oxide layer on the surface of the titanium biomaterial. The surface topography and the morphology of this oxide layer can be care-

fully controlled to obtain the desired characteristics. As a result of PEO, highly wear resistant and corrosion resistant surfaces are formed. Furthermore, the simultaneous incorporation of Ca, Si and P is possible in these oxide layers by the use of an appropriate electrolyte medium to conduct PEO. This PEO surface on titanium alloys becomes attractive for the osteoblasts to attach and proliferate to continue forming the peri-implant tissue [34]. Figure 4 is data for the degree of differentiation of hFOB 1.19 human fetal osteoblast cells on a variety of gamma TiAl substrates using an commercial Alkaline Phosphatase (ALP) Colorimeteric Assay kit. ALP expression was measured via Optical Density in a microplate reader. Clearly, the bioactive PEO coating inidcates a higher level of ALP expression compared to positive control and thermally oxidized surfaces. Furthermore, the same study reports the presence of nanopores which encourages the expression of collagen and bone sialoproteins and the availability of micropores which allow for the ingrowth of tissue results in the formation of the required tissue over the implant material. This increases the degree of osseointegration many-fold both from cell attachment on the PEO treated surface and the finger-like protrusion of the hard tissue into the pores.

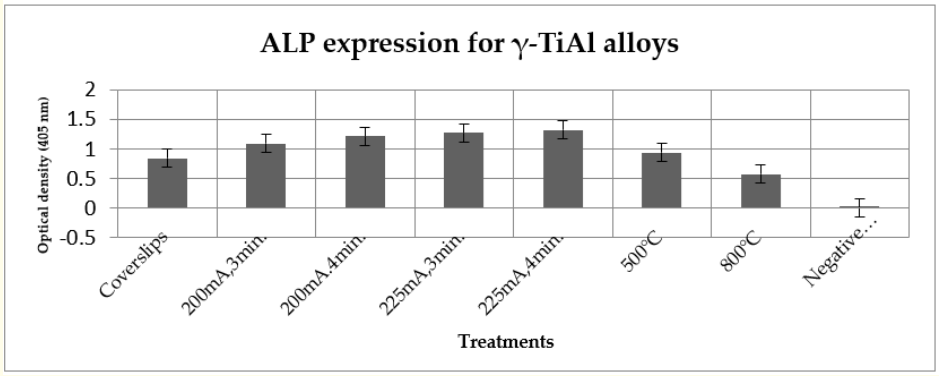

Figure 4

Figure 4 Alkaline phosphatase expression in human fetal obsteoblasts (hFOB 1.19) on gamma TiAl substrates subjected to plasma electrolytic oxidation (200 mA-3 min, 200 mA-4 min, 225 mA-3 min, 225 mA-4 min) and thermal oxidation (500 C and 800 C). Positive control (coverslip) and negative control (copper) are included for comparison. Adapted from [34].

Animal models

Two different animal models were studied with respect to the biocompatibility of gamma titanium aluminides. An initial rapid screening was carried out with Sprague-Dawley rats to check if indeed the Ti-48Al-2Cr-2Nb was biocompatible for repair and replacement applications in hard tissue. Based on the promising results that were obtained from the rat model and the data generated from the surface modification studies including cytocompatibiity, a larger sheep model was tested. Results of both animal model testing are detailed below.

Sprague-dawley rat model

Although it is not common to use the rat model for hard tissue applications, a screening at this smallest size category will provide a relatively rapid response regarding the suitability of the biomaterial for implant applications [9]. The surgical and implantation protocols were approved by the IACUC and all procedures were carried out according to strict controls. Sprague-Dawley rats, each weighing about 250 g each, were divided into control, sham and implant groups. In the sham group, holes were drilled through the femur without the placement of implants. For the implant group, single cortex (transcortical) implantation of 1 mm dia., 3 mm long cylinders of Ti-48Al-2Cr-

2Nb were made. Three such cylinders were placed in each of the right femurs of the animals in the implantation group corresponding to 45 days (acute), 90 days (sub-chronic) and 180 days (chronic) implantation periods. No adverse reactions or physical discomfort were observed in the animals. In fact, the Sprague-Dawley rats in the implantation group evolved in a normal manner similar to the control and sham groups. After each experimental period, the rats were sacrificed, and the implant regions were observed for any incompatibility. SEM, histology and serum analysis were carried out. Complete appositional bone growth over the implant was seen as early as 45 days. In the case of implants which were not placed flush with the cortex during surgery, a protruding callus formation was observed. Tissue growth was observed on lateral surface of cylinder implants. Layered growth of hard tissue was observed under the callus and this growth appeared to initiate from the Ti-48Al-2Cr-2Nb implant surface implicating the osseointegration of this biomaterial with the femoral tissue. Complete bone appositional growth occurred in the implant area after the 180-day implantation period. No vestiges of the implant were observed in the area. No tumors or any other abnormalities were seen in the peri-implant tissue. Overall, the Sprague-Dawley rat model indicated a complete acceptance of Ti-48Al-2Cr-2Nb as an implant material.

Figure 5 Scanning electron microscope images showing layered growth of bone tissue on gamma TiAl implant surface after 90 days, sub-chronic (left) and complete appositional bone growth on implant surface after 180 days, chronic (right). Adapted from [9].

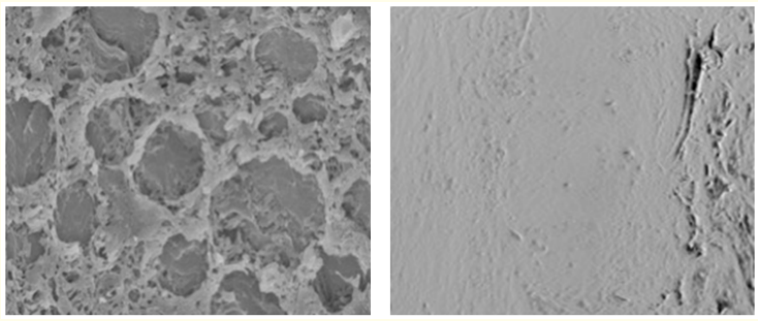

Figure 5

Sheep model

Three adult cross-bred sheep, 2 - 3 years old and weighing about 40 kg each were used for this animal model. Through this model, quantitative information regarding the osseointegration of PEO treated gamma titanium aluminide was solicited [35]. The implant screws, each with a length of 14 mm, a diameter of 3.5 mm and a core of 2.5 mm were placed in the right iliac wing of the two sheep. The third animal was used as control. All animal experiments were conducted according to IACUC approved protocol. A total of nine surgical screws were placed in random order, three each of Ti-6Al-4V, Ti-48Al-2Cr-2Nb with PEO treatment and Ti-48Al-2Cr-2Nb without PEO treatment in the ilia of the sheep. 3-month and 6-month implantation periods were used in this study. After the duration of implantation, the sheep ilia were carefully extracted, and torque removal tests were performed on the implanted screws. The average values of the maximum values of torque applied to remove these implanted screws are given in table 2. Clearly, the removal torque values of the PEO treated Ti-48Al-2Cr-2Nb samples are more than 1.5X the values for the untreated samples. This quantitatively reveals a greater degree of osseointegration of the PEO-treated gamma titanium aluminide when compared to the untreated alloy. Von Kossa staining of the histological samples obtained from the vicinity of the implant showed a larger degree of de novo bone formation for the PEO treated case. Analysis of trace metals in the blood serum samples also showed low concentrations which indicates implant stability [35]. High metallic element levels in the serum would be a cause for concern in terms of metal toxicity.

Titanium Alloy Type	Removal Torque (N.cm)	
	3 months	6 months
Ti6Al4V	96.03	113.42
Ti-48Al-2Cr-2Nb	92.65	99.03*
PEO-treated Ti-48Al-2Cr-2Nb	153.10	162.83

Table 2: *Average values of removal torque (n=2) for 3-month and 6-month implantations [35].*

**n=1.*

Discussion

A number of metallic biomaterials have been utilized in implant applications. Of these, the three most popular are the classic 316L stainless steel, a few Co-Cr based alloys and Ti-based alloys. While stainless steel has the necessary strength for hard tissue implant applications, its long-term corrosion stability is questionable. Another handicap is the high modulus of elasticity which is deemed to cause stress shielding in hard tissue implants. While stainless steels have the ability to form a passive oxide layer which is expected to provide corrosion resistance, it appears that in the human body, the material is subjected to localized pitting corrosion. Also, the fretting wear resistance of 316L stainless steel is poor, and the passive oxide layer does not reform over the affected area to endow the material with the necessary corrosion resistance. Cobalt-chromium alloys have better corrosion resistance in comparison to stainless steels and possess higher strength. Again, biocompatibility is suspect in the long-term and its use in active, younger patients where at least 30 - 40 years of implant stability without revision surgery is required, is not attainable.

The third group which has the best combination of properties for implant application are titanium-based alloys. Generally, Ti-based materials possess the enviable combination of properties for implant applications. Strength and corrosion resistance leading to biocompatibility is generally expected since titanium is known for being inert in many harsh environments, attributed to the formation of a tenacious oxide layer. Also, the elastic modulus of Ti-based alloys in much lower that either stainless steels or Co-Cr alloys and hence the problems related to stress shielding are expected to be minimal. Of course, the go-to material based on its track record in the aerospace industry and potential capability in hard tissue applications is Ti-6Al-4V. Ti-6-4, as it is popularly known has the required strength for implant applications. Unfortunately, the presence of vanadium in the alloy has been reported to cause toxicity leading to cell death in the peri-implant region with ensuing implant loosening and failure [30]. Commercially pure titanium or CP-Ti while without the issue of vanadium toxicity, falls short of the excellent corrosion resistance or associated wear resistance of the other Ti-based alloys. While expected to possess excellent biocompatibility, CP-Ti does not have the required mechanical properties for robust structural applications in the human body.

Based on earlier reports and the experimental evidence provided in the earlier sections, gamma titanium aluminide appears to have adequate strength and fatigue resistance to be applied in the body in the form of structural implants. Further engineering of its surface through oxidation not only increases corrosion and wear resistance considerably but provides the luxury through biocompatibility of a vanadium-free oxide layer which encourages bone morphogenesis making it an attractive material for hard tissue implants. From earlier studies, it appears that the plasma electrolytic oxidation (PEO) process in a simulated body fluid solution is extremely beneficial in facilitating the growth of de novo bone tissue over gamma titanium aluminide. The Ca and P elements that are incorporated into the oxide matrix and the morphology of the nano-scale pores in the PEO layer further encourage the formation of bone tissue to enhance the degree of osseointegration. A rigorous analysis based on experimental evidence suggests that besides the basic requirements of strength and fatigue resistance, many hard tissue applications require excellent corrosion and associated wear resistance. The potential implant material must have the capacity to form a tenacious oxide layer which on being removed under fretting conditions must quickly form an

effective oxide layer as a replacement. Besides, this desired oxide layer must be osteoconductive by being bioactive in encouraging the deposition of the components of de novo bone eventually leading to its effective formation. Figure 6 shows a concept diagram of the process of osseointegration. In the case where the implant is unable to develop a feasible and tough oxide layer, it is not possible for de novo bone to deposit over bare metal merely as a result of chemical incompatibility. However, bone deposits can form chemical bonds with ceramic layers in the case where a bioactive and tough oxide layer can form on the substrate metal. When these oxides are tenacious, have sufficient strength and are conducive to bone deposition, trabecular bone can grow on this surface and fuse with the bone growing from the exisitng tissue leading to enhanced osseointegration. Additionally, if there is appropriate porosity for the bone to grow into the pores, a mechanical connection, over and above the chemical bond, is established. Such a de novo bone layer is able to meld to the peri-implant tissue easily leading to successful implant osseointegration.

Figure 6 Concept diagram of the implant-hard tissue interface. (A) trabecular bone growth is possible only on the exisiting bone surface in the absence of a bioactive oxide layer on the metallic implant surface and (B) enhanced osseintegration can be achieved when a bioactive oxide layer can be grown on the metallic implant surface resulting in trabecular bone growth on implant surface which can bond easily with growth on existing bone.

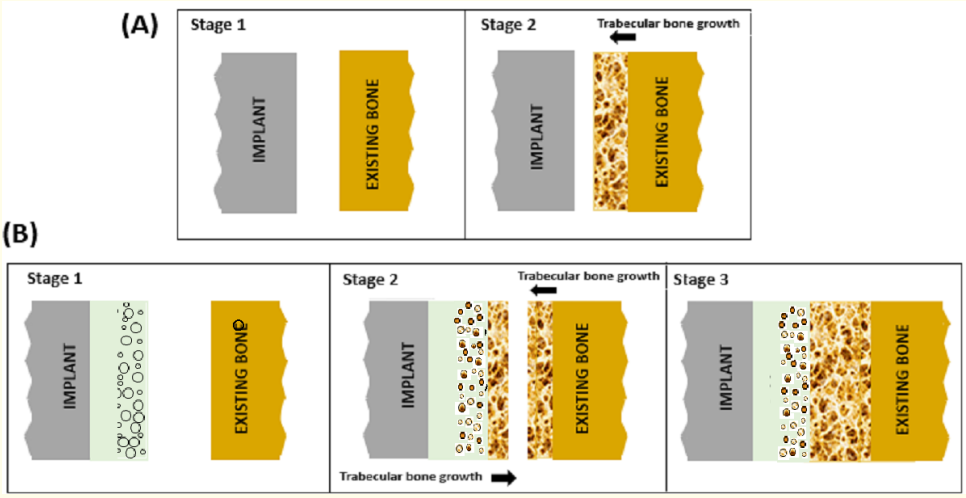

Figure 6

Concluding Remarks

The experimental evidence presented from fundamental laboratory experiments in corrosion, wear testing, cytocompatibility to the more complex animal models demonstrate effectively that gamma titanium aluminide has the characteristics and hence the potential as an excellent implant material. It has similar or superior biocompatibility compared to Ti-6Al-4V and is not linked with vanadium toxicity. If advantage is taken of the possibility of growing a bioactive titanium oxide layer through plasma electrolytic oxidation, gamma titanium shows excellent potential to be used in implant applications.

Acknowledgments

The author is grateful to the Office of Naval Research Global for support during his tenure as Science Director in São Paulo, Brazil.

Bibliography

1. Bumps ES., *et al*. "Titanium-aluminum System". *AIME Trans* 194 (1952): 609-614.
2. Ogden HR., *et al*. "Mechanical properties of high purity Ti-Al alloys". *AIME Trans* 197 (1953): 267-272.
3. Kim YW and Dimiduk DM. "Progress in the understanding of gamma titanium aluminides". *JOM* 43 (1991): 40-47.
4. Kelly TJ., *et al*. "Effect of elevated temperature exposure on cast gamma titanium aluminide (Ti-48Al-2Cr-2Nb)". *Scripta Metall Mater* 30 (1994): 1105-1110.
5. Bartolotta P., *et al*. "The use of cast Ti−48Al−2Cr−2Nb in jet engines". *JOM* 49 (1997): 48-50.
6. Stroosnijder MF., *et al*. "Oxidation behaviour of TiAl-based intermetallics – influence of heat treatment". *Materials Science and Engineering: A - Journal* 239-240 (1997): 842-846.
7. Loria EA. "Gamma titanium aluminides as prospective structural materials". *Intermetallics* 8 (2000): 1339-1345.
8. Kyziol K., *et al*. "Structure, characterization and cytotoxicity study on plasma surface modified Ti-6Al-4V and g-TiAl alloys". *Chemical Engineering Journal* 240 (2014): 516-526.
9. Castañeda-Muñoz DF., *et al*. "Bone tissue reaction to Ti–48Al–2Cr–2Nb (at.%) in a rodent model: a preliminary SEM study". *Journal of Materials Science: Materials in Medicine* 18 (2007): 1433-1438.
10. Som I., *et al*. "Thermally oxidized electron beam melted g-TiAl: In vitro wear, corrosion and biocompatibility properties". *Journal of Materials Research* 33 (2018): 2096-2105.
11. Huiskes R., *et al*. "The relationship between stress shielding and bone resorption around total hip stems and the effects of flexible materials". *Clinical Orthopaedics and Related Research* 274 (1992): 124-134.
12. Winter W., *et al*. "Micromotion of dental implants: basic mechanical considerations". *Journal of Medical Engineering and amp; Technology* (2013): 265412.
13. Szmukler-Moncler S., *et al*. "Timing of loading and effect of micromotion on bone-dental implant interface: review of experimental literature". *Journal of Biomedical Materials Research* 43 (1998): 192-203.
14. Branemark R., *et al*. "Osseointegration in skeletal reconstruction and rehabilitation". *Journal of Rehabilitation Research and Development* 38 (2001): 175-181.
15. Mei D., *et al*. "Selecting medium for corrosion testing of bioabsorbable magnesium and other metals – a critical review". *Corrosion Science* 171 (2020): 108722.
16. Pearce AI., *et al*. "Animal models for implant biomaterial research in bone: a review". *European Cells and Materials - DOAJ* 13 (2007): 1-10.
17. Wancket LM. "Animal models for evaluation of bone implants and devices: comparative bone structure and common model uses". *Veterinary Pathology* 52 (2015): 842-850.

18. McGovern JA., *et al*. "Animal models for bone tissue engineering and modelling disease". *Disease Models and Mechanisms* 11 (2018): dmm033084.

19. Li Y., *et al*. "Bone defect animal models for testing efficacy of bone substitute biomaterials". *Journal of Orthopaedic Translation* 3 (2015): 95-104.

20. Gomes PS and Fernandes MH. "Rodent models in bone-related research: the relevance of calvarial defects in the assessment of bone regeneration strategies". *Lab Animal* 45 (2011): 14-24.

21. Sommer U., *et al*. "In vitro and in vivo biocompatibility studies of a cast and coated titanium alloy". *Molecules* 25 (2020): 3399.

22. Spece H., *et al*. "A systematic review of preclinical in vivo testing of 3D printed porous Ti6Al4V for orthopedic applications, part I: Animal models and bone ingrowth outcome measures". *Journal of Biomedical Materials Research* 109 (2021):1436-1454.

23. Frosch S and Buchhorn GH. "Considerations on the animal model and the biomechanical test arrangements for assessing the osseous integration of orthopedic and dental implants". *MethodsX* 8 (2021): 101352.

24. Nuss KM., *et al*. "An animal model in sheep for biocompatibility testing of biomaterials in cancellous bones". *BMC Musculoskeletal Disorders* 7 (2006): 67.

25. Dowd JE., *et al*. "Aseptic loosening in uncemented total hip arthroplasty in a canine model". *Clinical Orthopaedics and Related Research* s 319 (1995): 106-121.

26. Bédard P., *et al*. "Innovative human three-dimensional tissue-engineered odels as an alternative to animal testing". *Bioengineering* 7 (2020): 115.

27. Rivera-Denizard O., *et al*. "Biocompatibility studies of human fetal osteoblast cells cultured on gamma titanium aluminide". *Journal of Materials Science: Materials in Medicine* 19 (2008): 153-158.

28. Delgado-Alvarado C and Sundaram PA. "Corrosion evaluation of Ti–48Al–2Cr–2Nb (at.%) in Ringer's solution". *Acta Biomaterialia* 2 (2006): 701-708.

29. Bello SA., *et al*. "In vitro evaluation of human osteoblast adhesion to a thermally oxidized gamma-TiAl intermetallic alloy of composition Ti-48Al-2Cr-2Nb (at.%)". *Journal of Materials Science: Materials in Medicine* 21 (2010): 1739-1750.

30. Costa BC., *et al*. "Vanadium ionic species from degradation of Ti-6Al-4V metallic implants: In vitro cytotoxicity and speciation evaluation". *Materials Science and Engineering C* 96 (2019): 730-739.

31. Ramos-Saenz CR., *et al*. "Tribological properties of Ti-based alloys in a simulated bone-implant interface with Ringer's solution at fretting contacts". *Journal of the Mechanical Behavior of Biomedical Materials* 3 (2010): 549-558.

32. Liu W., *et al*. "Surface modification of biomedical titanium alloy: micromorphology, microstructure evolution and biomedical applications". *Coatings* 9 (2019): 249-272.

33. Lara Rodriguez L., *et al*. "Plasma electrolytic oxidation coatings on γTiAl alloy for potential biomedical applications". *Journal of Biomedical Materials Research – Part B: Applied Biomaterials* 102 (2014): 988-1001.

34. Santiago-Medina P., *et al*. "The effects of micro arc oxidation of gamma titanium aluminide surfaces on osteoblast adhesion and differentiation". *Journal of Materials Science: Materials in Medicine* 25 (2014): 1577-1587.

35. Richiez-Nieves P*., et al*. "A sheep model for the osseointegration of PEO-treated gamma titanium aluminide". *European Journal of Dental and Oral Health* 1 (2020).

Volume 21 Issue 3 March 2022