

Bone tissue reaction to Ti–48Al–2Cr–2Nb (at.%) in a rodent model: a preliminary SEM study

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Abstract A variety of metals have been used to replace the skeletal framework of human beings. Gamma titanium aluminide (γ TiAl) has been recently developed as a prospective material for turbine applications. In this preliminary study, the potential of γ TiAl as a biomaterial was evaluated using an in vivo rat model. Sprague–Dawley rats were implanted with γ TiAl cylinders in the femur and observed for an experimental period lasting up to 180 days. The rats were sacrificed after periods of 45, 90 and 180 days. The femurs with the γ TiAl implants were extracted and examined using scanning electron microscopy (SEM). Normal bone growth processes were observed as early as 45 days after γ TiAl cylinder implantation. No signs of rejection of the implant metal were observed. In fact, a layered bone growth was observed on the implant metal surface. The bone–metal interface showed signs of tissue growth from original bone to the metal surface. γ TiAl appears to elicit a normal bone tissue reaction and hence, has potential as a metallic implant material.

1 Introduction

Various metallic materials have been used in humans for repair and replacement of the body's skeletal framework [1]. Stainless steel and cobalt chromium (Co–Cr) alloys were the first materials successfully used inside the body for fracture fixation [2, 3]. Although stainless steels have the strength and ductility for proper functioning of the implant [1], they are susceptible to corrosion in the biological environment [4]. The high elastic moduli of the Co–Cr implants have been reported to cause site osteoporosis and stress-shielding at the femoral stem/neck interface in hip replacements [4]. Reports on the use of Co–Cr–Mo alloy (also known as F-75) implants in rats for prolonged periods have indicated site inflammation, and in some cases, mortality due to the release of corrosion products [5]. There is concern that the corrosion of cobalt–chrome in the wet, salty surroundings of the human body may be sending toxins streaming throughout the body, possibly leading to malignant transformation of cells [6]. Skin conditions, such as dermatitis, have been reported from exposure to nickel [7].

Among the metals currently used in biomedical applications, titanium alloys appear to be the most popular as implant materials because of their low density, excellent mechanical–chemical properties and outstanding biocompatibility [6, 8, 9]. The most important Ti-based materials currently used in bone repair and replacement are Ti6Al4V and commercially pure (cp) titanium [10]. They have high strength in relation to their relatively low weight. A titanium alloy implant has a stiffness of less than half of that of stainless steel or Co–Cr alloy, which reduces the effects

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of stress shielding. Its constituents provide excellent corrosion resistance, but it does suffer from relatively low fracture toughness and poor wear properties [6]. Ti alloys have poor shear strength and cause seizing because of high coefficients of friction, both in bone–metal and metal–metal interfaces [1, 11].

Recently, a Ti-based material, gamma titanium aluminide (γ TiAl or gammalloy) has been developed for aerospace applications. γ TiAl alloys are currently being developed for applications in the automotive and aerospace industries such as valves, rotors and turbine blades of supersonic jet engines [12–14]. γ TiAl has been extensively studied for its mechanical properties, low density, and high temperature oxidation resistance [12–18]. In spite of these excellent mechanical–chemical properties, γ TiAl has never been considered as a biomaterial in the scientific literature.

The purpose of this research was to carry out a preliminary study of the bone tissue response to γ TiAl alloy in vivo, using a rat model. Rat femurs were implanted with three cylinders of gamma titanium aluminide and the bone tissue response was evaluated qualitatively using scanning electron microscopy. To the best of our knowledge, there is no information in current literature regarding the bone tissue response to γ TiAl in animals or humans.

2 Materials and methods

The γ TiAl used in this study is Ti–48Al–2Cr–2Nb (at. %). It has a predominantly lamellar microstructure with a small volume fraction of single-phase gamma grains. The typical microstructure of this alloy is given in Fig. 1. The implants used were cylinders 2.8 mm long and 1.0 mm in diameter. This length was chosen

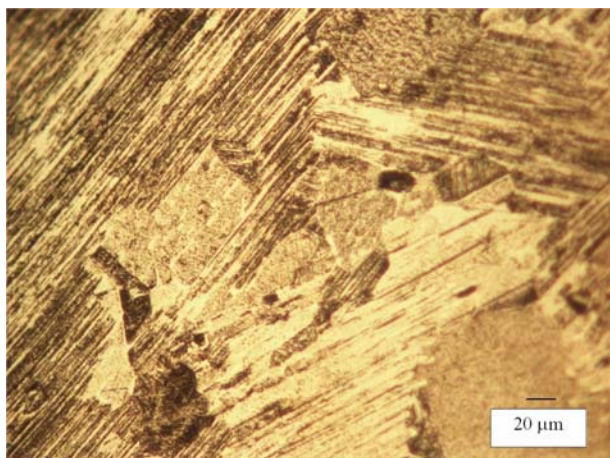


Fig. 1 Microstructure of Ti–48Al–2Cr–2Nb (at. %)

to allow the implant to reside in one cortex and the medulla without excessive protrusion beyond the next cortex and the periosteum. Also, this diameter of the implant was selected as not to cause impending fracture during the implantation and follow-up since three cylinders were implanted in each femur. The material was produced from γ TiAl powder by hot isostatic pressing at 1,000°C and received in the form of 25-mm diameter rods about 175–200 mm long. The cylinders were machined from the as-received rods using electro-discharge machining (EDM). These samples were ground with silicon carbide grinding paper (grit #320) resulting in circumferential texture on the sample surface. These ground implants were cleaned and sterilized in a water vapor autoclave at 121°C and 15 psi for 1 h prior to implanting in the rats.

A total of 12 healthy Sprague–Dawley male rats, based on the ASTM protocol F 981-93, weighing approximately 250 g each were used for the study. The rats were divided into three groups: acute (45 days), sub-acute (90 days) and chronic (180 days). Each group in turn consisted of four (4) rats: one (1) control, one (1) placebo and two (2) which were implanted with the Ti–48Al–2Cr–2Nb metal. Control rats were used only for comparison and hence were not subjected to any surgical procedure. Placebo rats were surgically invaded as described below but no implants were placed in these rats. NIH guidelines for the care and use of laboratory animals (NIH publication # 85-23 Rev. 1985) were strictly observed.

The rats were anesthetized with pentobarbital, the dose being approximately 0.2 mL of 65 mg/mL solution of sodium pentobarbital per 250 g of rat. The anesthetic was administered intraperitoneally. The rats in the placebo (surgically invaded but not implanted) and implantation (surgically invaded and implanted) groups were placed on a surgical table. For the purpose of this study, only the right thigh was shaved and scrubbed with butadiene and 70% isopropyl alcohol several times. The right femur was exposed with a longitudinal dissection of approximately 15 mm. Holes 1 mm in diameter were drilled in the proximal, medial and distal parts perpendicular to the long axis of the femur at 10 mm separations to avoid fracturing the femur. The sterilized cylindrical implants of γ TiAl were then placed through the hole perforated in the cortex and into the bone marrow. These implants did not penetrate the cortex on the opposite side. The surgical area was sutured by layers and then cleaned. The rats were allowed to recover in their respective cages. The animals were carefully observed for signs of infection and/or foreign body reaction for the length of the experimental period.

At 45 days (acute period) the first group of four rats was sacrificed. The same procedure was carried out with the second group at 90 days (sub-acute period) and with the third group at 180 days (chronic period). After sacrificing the rats, the femur of interest (right femur) was isolated and preserved for further analysis.

The extracted femurs were fixed in 10% formalin for 7 days. These tissues were then washed in distilled water in three changes of 15 min each and glutaraldehyde for 1 day to eliminate the formalin and avoid dehydration of the tissue. Each femur was cleaned thoroughly to remove surface contaminants and three lateral sections of 5-mm long pieces of the femur including the implants were obtained. Each sectioned specimen containing one implant each was placed in fixative formalin for 3 days at room temperature. The fixative was then discarded and the specimen was rinsed twice in sodium phosphate buffer for 15 min each. Each specimen was further dehydrated in ascending ethanol: 10, 25, 50, 75 and 95% for 10 min each and in 100% with three changes of 10 min each. Specimen desiccation was carried out using critical point drying. Each specimen was mounted onto the scanning electron microscope (SEM) stub with silver paint and quickly stored in a clean desiccator. The sample was coated with gold in a sputter-coater and taken to the SEM for examination. The tissue response to the presence of the γ TiAl cylinders was examined. The differences between the implants and the influence of time were analyzed.

3 Results

In this preliminary research, gamma titanium aluminide (γ TiAl) implants were made in an *in vivo* rat model to study bone tissue response to the presence of this material. Evident bone growth surrounding the implants without any gap between bone and metal was observed in all the implants showing complete acceptance of the metal. Figure 2 show the typical implant in the femur. Figure 3 shows growth of compact bone over the γ TiAl implant after 45 days. The circular cross-section of the implant can be recognized under the compact bone layer covering the metal implant. This appositional growth of bone tissue over the implant indicates that there is no adverse reaction to the γ TiAl alloy implant. It should be mentioned that in the case where the implant surface was not at the same level as the cortical surface of the femur, a protruding callus was observed. When the implant surface was flush with the cortex, a smooth layer of cortical tissue grew over the implant.

In Fig. 4, the implant surface after 90-day duration shows no foreign body reaction of the bone to the

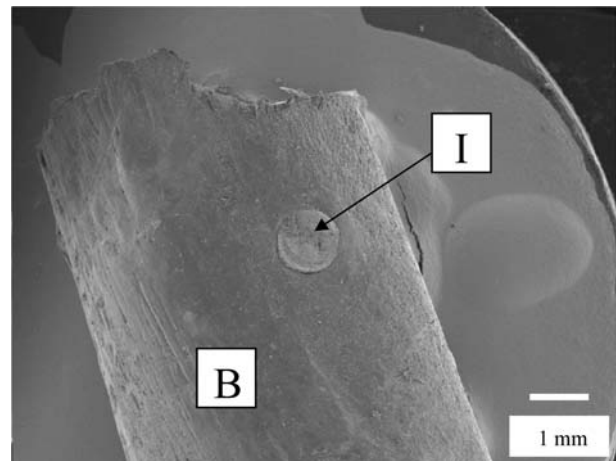


Fig. 2 The γ TiAl implant in the rat femur. Bone (B), Implant (I)

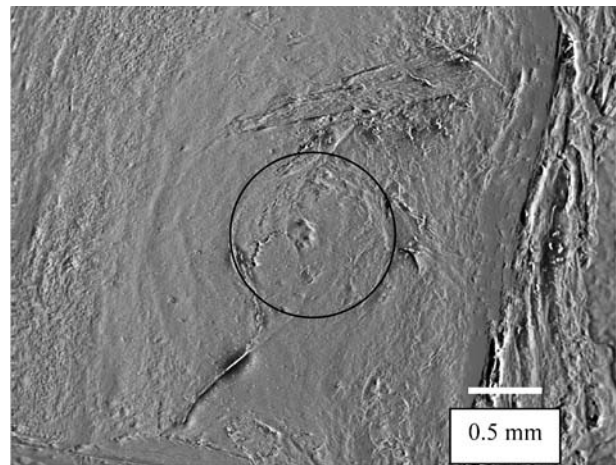


Fig. 3 Cortical bone tissue covering the implant completely after 45 days. Circle shows profile of implant under the bone tissue

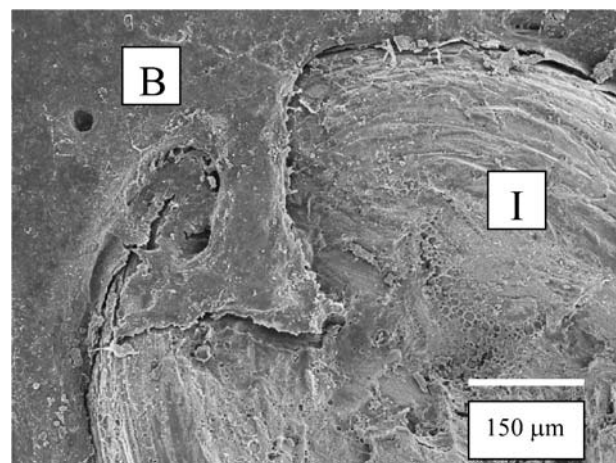


Fig. 4 Top surface of an implant of 90-day duration where the callus was scraped off to observe bone growth. Bone (B), Implant (I)

presence of the metal. In this case, the callus formed was scraped off to observe the inner layers. Figure 5 shows this same metal implant surface at a higher magnification, where the bone tissue over the implant surface is found to be of a cancellous nature and appears to be in the process of forming compact bone. The cross-section of another femur with a lateral view of the implant with 90-day implant longevity is seen in Fig. 6. It is observed that the implant resides in one cortex and bone marrow without penetration into the opposite cortex. The γ TiAl alloy implant is absorbed completely in the bone marrow. Normally an immediate reaction of the cells in the biological medium to a foreign body is expected in the bone marrow. However

from this figure, it is clear that there is no adverse tissue response since the bone marrow has completely enveloped the metal implant without any foreign body reaction. Examination of the 180-day implant (Fig. 7) showed that the base γ TiAl alloy was completely covered with cortical bone tissue. In all cases, no inflammation, tumors or other adverse reactions were observed on the implanted femurs. The lack of the evidence of inflammation indicates that the tissue has adjusted to the presence of the implant. A large degree of mineralization has taken place to establish the anatomical and mechanical continuity of the bone–metal interface for all the implantation time periods. This is clearly observed in Figs. 8 and 9 which

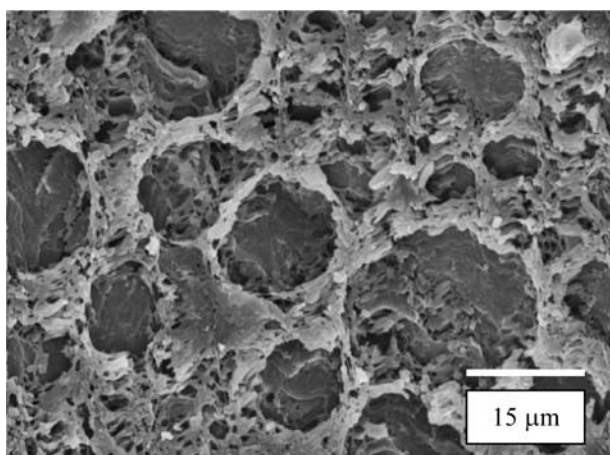


Fig. 5 Surface region from Fig. 4 indicating layered bone growth over metal implant after callus removal

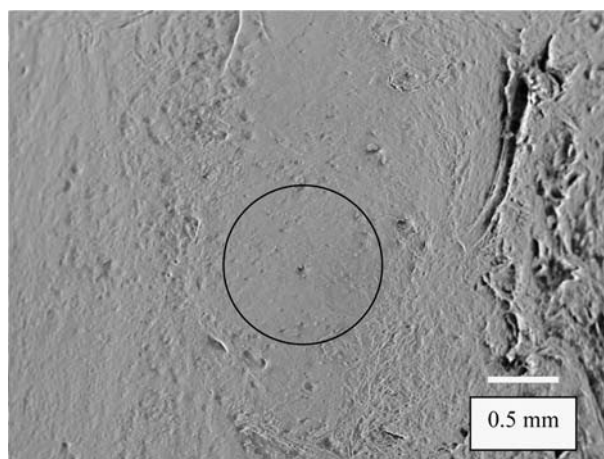


Fig. 7 Cortical bone tissue covering the implant after 180 days. Circle corresponds to profile of implant under the bone tissue

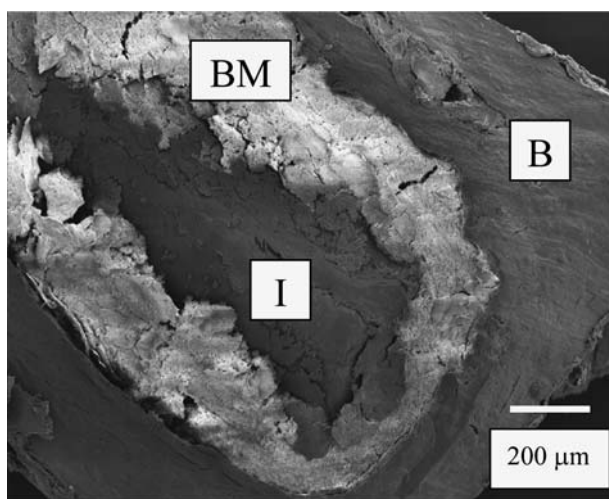


Fig. 6 Cross-section of femur in the area of implant showing bone tissue growth on gamma TiAl alloy. Note, the bone marrow (BM) is seen as lighter contrast compared to the cortical bone (B) and the metal implant (I)

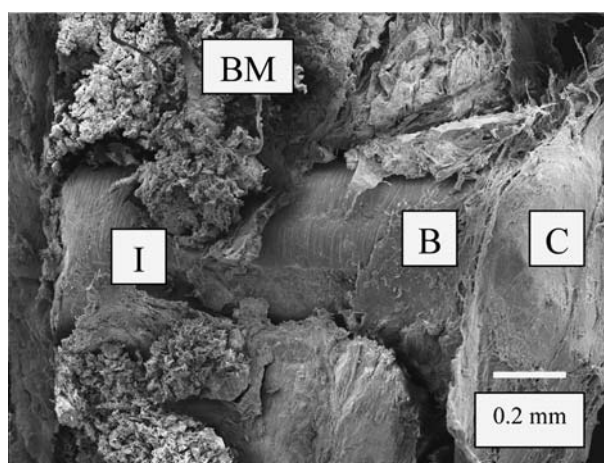


Fig. 8 Femoral cross-section showing longitudinal view of implant (I) after 45 days surrounded by bone marrow (BM). The callus (C) was maintained intact to obtain the SEM image. Bone tissue (B) growth is seen on the implant with fibrous interface

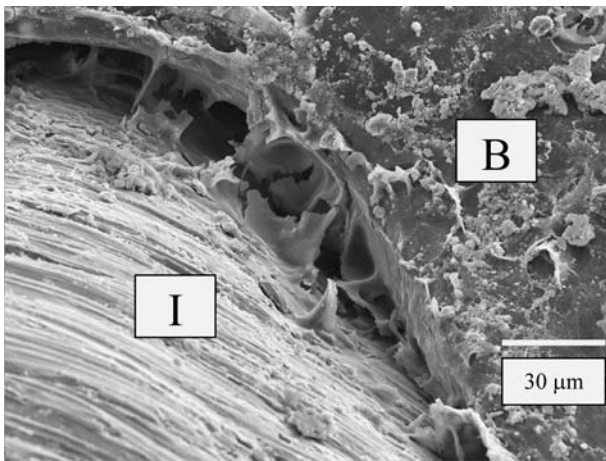


Fig. 9 Bone–metal implant interface showing attachment between original femur and newly growth bone tissue on implant surface. Implant duration was 90 days. Bone (B), Implant (I)

demonstrate the bone–metal interface indicating evidence of attachment of tissue from the femur surface to newly formed tissue on the metal implant surface after implant duration of 45 and 90 days, respectively.

4 Discussion

One aspect of biocompatibility is response of tissue to the presence of an implant. Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific situation [19]. In this case, a biocompatible material should exist in contact with bone tissue without causing damage or necrosis to the latter. This material should not impede in any way the proliferation and growth of bone cells as part of the normal growth process of bone. In addition, a biocompatible material shows osseointegration where apposition of the bone over the implant occurs without intervening fibrous tissue. From the results of this study it is clear that bone tissue did not reject the γ TiAl implant. No signs of necrosis, lesions or other abnormalities were visible in the implanted rats during the whole observation period of 6 months.

When a hole is drilled to introduce the implant into the femur in the rat model, damage is caused to the cortex and medulla. Rupture of capillaries in the vicinity is also a consequence. This damage produces an acute inflammation and generates healing reactions in the adjacent tissue [20, 21]. During the healing process, after fracture or damage to bone tissue, callus development occurs by the formation of a woven matrix which slowly transforms to compact bone. This process appears to be the rat femoral bone tissue

response to the presence of the γ TiAl implant. Furthermore, the appositional growth of cortical bone over the γ TiAl implant indicates normal tissue response. This is also supported by the complete enveloping of the implant by the bone marrow as seen in Fig. 6. The fact that tissue growth occurs in the bone–metal interface from the metal surface to the original tissue in the femur (Fig. 9) again is clear evidence of the potential biocompatibility of γ TiAl.

In summary, this preliminary study indicates that gamma titanium aluminide has the potential as a candidate material for bone repair and joint replacement due to the observed normal tissue response. Clearly, other rigorous studies on gamma titanium aluminide in a biological fluid and further testing in animal models must be carried out before recommending this material for hard tissue implant application.

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