Histomorphological and Histomorphometric Analyses of Grade IV Commercially Pure Titanium and Grade V Ti-6Al-4V Titanium Alloy Implant Substrates: An *In Vivo* Study in Dogs

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Purpose: *To evaluate the bone response to grade IV commercially pure titanium (G4) relative to Ti-6Al-4V (G5).*

Materials and Methods: *Implant surface topography was characterized by optical interferometry and scanning electron microscopy (SEM). Thirty-six implants (Signo Vinces, n = 18 per group) were installed in the radius of 18 dogs. The animals were killed at 1, 3, and 6 weeks, resulting in 6 implants per group and time* in vivo *for bone morphology, bone-to-implant contact (BIC), and bone area fraction occupancy (BAFO) evaluation.*

Results: *SEM depicted a more uniform topography of G4 than G5. Surfaces were statistically homogeneous for Sa, Sq, and Sdr. At 1 week, new bone formation was observed within the healing connective tissue in contact with the implant surface. At 3 weeks, new bone in direct contact with the implant surface was observed at all bone regions. At 6 weeks, the healing chambers filled with woven bone depicted an onset of replacement by lamellar bone. No significant effect of substrate was detected. Time presented an effect on BIC and BAFO (*$P < 0.001$*).*

Conclusion: *Both titanium substrates were biocompatible and osseoconductive at the bone tissue level. (Implant Dent 2016;25:650–655)*

Key Words: dental implants, titanium, alloy, osseointegration

Historically, prosthetic replacement of lost dentition has been always a matter of concern.[1] Different materials have allowed the development of new prosthetic and reconstructive techniques, and more recently, the use of titanium enhanced the process of osseointegration.[1–3]

The first description of a biologic compatible response between bone and titanium dates from 1940 when Bothe et al[4] observed that titanium was not only well tolerated but most importantly that there was a tendency of bone growth when in contact with it. Nearly a decade later, Leventhal[5] described good biocompatibility and gradual integration between bone and titanium. Subsequently, a Swedish group further characterized the osseointegration phenomenon as a direct structural and functional connection between living bones and the surface of an implant at the optical microscopy level.[6] The same group also suggested over the years that the establishment of osseointegration is highly dependent on multiple parameters that include implant material, implant design, implant processing (sterilization and cleanliness), status of the bone that will receive the implantable device, surgical technique, implant loading conditions, among others.[7]

Presently, the American Society for Testing and Materials defines at least 39 different compositions for titanium alloys that include commercially pure (CP) grades and titanium

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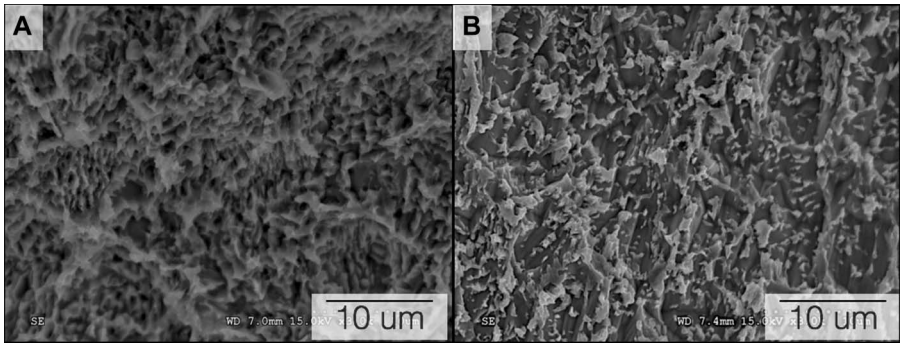

Fig. 1. SEMs for the (**A**) G4 and (**B**) G5 implant surfaces depicting that the same surface production procedure resulted in different surface morphologies which presented numerically similar texture metrics as presented in Table 1.

Table 1. IFM Measurements for the Different Substrate Alloys' Surfaces

	Sa (μm)	Sq (μm)	Sdr (×100%)
Grade IV	0.488	0.580	0.249
SD	0.131	0.172	0.072
Grade V, Ti-6Al-4V	0.454	0.559	0.277
SD	0.080	0.101	0.075

Surfaces from both G4 and G5 substrates are statistically homogeneous for the parameters Sa, Sq, and Sdr.

alloys presenting higher content of either alpha phase or beta phase stabilizers.[8] The mechanical and physical properties of these materials differ significantly mainly in terms of yield strength, ultimate tensile strength, and fatigue strength,[9] which eventually implies in differences in the probability of survival of restored dental implants.[10]

In the dynamic oral environment, high mechanical properties allow implant and connections to reliably survive occlusal loads, minimizing device failure due to fracture.[9,10] Although increasing the fatigue resistance of implantable devices is of interest from a load bearing capability standpoint, such increases are usually obtained along with an increase in the alloy's elastic modulus. However, such increase in elastic modulus further increases the bone/implant elastic modulus mismatch and force transmission to the implant surrounding the bone, potentially resulting in bone resorption due to either bone overloading or the overall stiffening of the implant/bone biomechanical system, or underloading that potentially leads to bone stress shielding.[11] Despite the lower mechanical properties presented by the different grades of CP (tuned by carefully controlling the amount of interstitial alloying content within the alpha titanium lattice) relative to other biocompatible alloys such as Ti-6Al-4V, all these alloy types have been extensively used in a variety of craniomaxillofacial and orthopedic scenarios, presenting adequate clinical success as anchor devices.[9] The osseointegration achievement for these different alloys is strongly supported by a plethora of preclinical laboratory models.[12–14] However, studies directly comparing screw for implants made of different alloy substrates presenting similar surface finish and texture, cleaning and sterilization procedures that are nested within the same translational animal subject are sparse in the dental and orthopedic literature. Thus, the aim of this study was to histomorphologically/histomorphometrically evaluate the bone response to grade IV CP titanium (UNS R50700) and grade V titanium alloy Ti-6Al-4V (UNS R56400—6% aluminum, 4% vanadium) implants with similar surface finish subjected to the same cleaning and sterilization procedures in a beagle dog model.

MATERIALS AND METHODS

Forty-two dental implants (4.6 mm diameter × 10 mm length) tapered dental implants (Duo model; Signo Vinces, Campo Largo, Brazil) were used in this study. The study groups consisted of 21 grade V Ti-6Al-4V titanium alloy substrate (G5) (UNS R56400—6% aluminum, 4% vanadium) implants, and the control group consisted of another 21 grade IV CP titanium implants (G4).

For surface texture characterization, 3 implants per surface condition were used. Scanning electron microscopy (SEM) (Philips XL 30; Eindhoven, the Netherlands) was performed at various magnifications under an acceleration voltage of 15 kV. Surface roughness was evaluated in different implant surfaces by optical interferometry (IFM) (Phase View 2.5; Paris, France) at the flat region of the implant cutting edges (3 measurements per implant). Sa (arithmetic average high deviation), Sq (root mean square), and Sdr (developed surface ratio) parameters were determined. A filter size of 250 μm × 250 μm was used for a total of 15 measurements per surface.

The laboratory *in vivo* surgical model was conducted under the approval of Ethics Committee on animal research of the École Nationale Vétérinaire (Paris, France).

For the laboratory *in vivo* model, 18 healthy male beagle dogs presenting stabilized bone growth (approximately 1.5 years) were acquired and housed for a period of 2 weeks in the animal facility for acclimatization. The surgical site selected for implant placement was the radius diaphysis, and 2 implants, one of each group, were installed in each subject with interchanged fixture position (proximal-distal) between animals for a balanced number of devices per group and time *in vivo*.

All surgical procedures were performed under general anesthesia. Preanesthetic care administration included intramuscular (IM) atropine sulphate (0.044 mg/kg) and xylazine hydrochloride (8 mg/kg). General anesthesia was performed by IM ketamine administration (15 mg/kg).

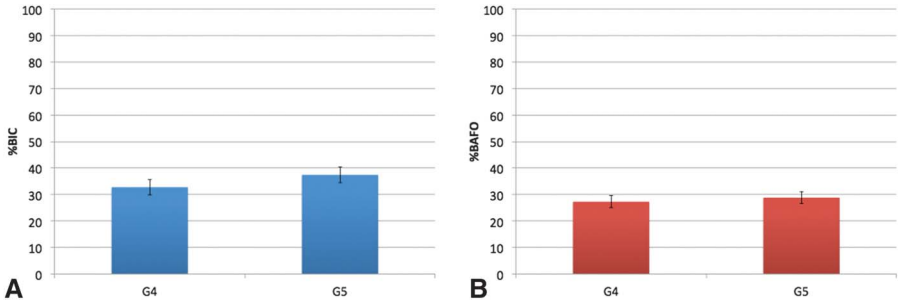

Fig. 2. Summary of statistical results (mean ± standard error) for (**A**) BIC and (**B**) BAFO depicting that no significant effect of titanium substrate was detected for BIC and BAFO collapsed over time.

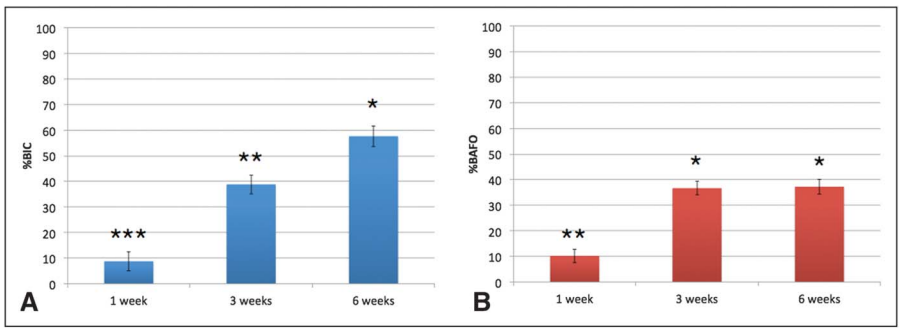

Fig. 3. Summary of statistical results (mean ± standard error) for (**A**) BIC and (**B**) BAFO depicting that a significant increase in both osseointegration indicators occurred as time elapsed *in vivo* for BIC and between 1 and 3 weeks for BAFO. The number of asterisks depicts statistically homogeneous groups.

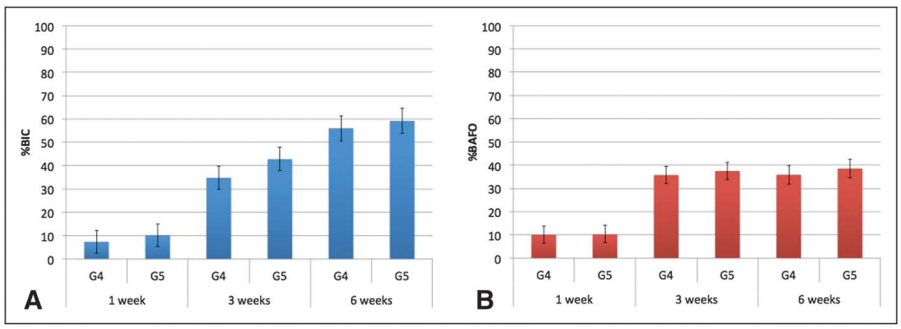

Fig. 4. Summary of statistical results (mean ± standard error) for (**A**) BIC and (**B**) BAFO as a function of implant substrate and time *in vivo*. Although BIC values were in general higher for G5 substrate implants, statistical analysis failed to demonstrate a significant effect of the G5 alloy on BIC at all times *in vivo*. No significant differences in BAFO were observed between alloys at any time *in vivo*.

Antisepsis of the surgical and adjacent areas was performed with an iodine-based solution followed by trichotomy. A 5-cm incision was made, followed by musculature dissection and periosteum exposure. Two osteotomies were performed at least 3 cm apart from each other. The drilling protocol was performed under copious saline irrigation. The sequence was initiated with a 2 mm diameter pilot drill at 1200 rpm, reaching a depth of 10 mm. Subsequent instrumentation was performed after the sequence determined by the manufacturer (3.8 and 4.6 mm drills). Implants were inserted using a manual ratchet system.

After surgical installation, each fixture received a cover screw to prevent bony callus formation that could otherwise result in potential bone/implant interface damage during surgical removal. Primary wound closure was obtained. Suture was performed using 4-0 Vicryl for inner layers and 3-0 nylon for the most superficial layer.

Postoperative care included administration of antibiotics (Penicillin, 20.000 UI/kg) and anti-inflammatories (Ketoprofen, 1 mL/5 kg) for a period of 48 hours.

Euthanasia was performed at the first, third, and sixth week postintervention in groups of 6 animals, resulting in 6 implants per group and time *in vivo*. After euthanasia, the upper third of the limb was exposed by gentle dissection and soft tissue was removed. An initial clinical evaluation of implant stability was performed. If clinical absence of implant stability was noted, the fixture was excluded from further analysis. The radii were then sectioned so that a portion containing the 2 implants was obtained. This sample was later divided into 2 separate blocks containing each one an implant at its center.

The samples were then fixed in 10% formalin for 5 days and were sequentially dehydrated in alcohol solutions and later embedded in an acrylate-based resin (Technovit 9100; Kulzer GmbH, Germany). A precision saw (Isomet 1000; Buehler, Illinois) was used to obtain a central section of each sample with an approximate thickness of 300 μm. These resulting sections were glued to acrylic sheets and gradually polished with a silicone-based carbide abrasive paper. An increasingly finer grit sequence—grain 400, 600, 800.1200, 1400 (Buehler)—was used until a final thickness of approximately 30 μm was obtained, allowing the performance of a Stevenel and Van Gieson staining technique.

The percentage of bone-to-implant contact (BIC) and bone area fraction occupied (BAFO) were determined at a ×50 magnification (DM4000; Leica,

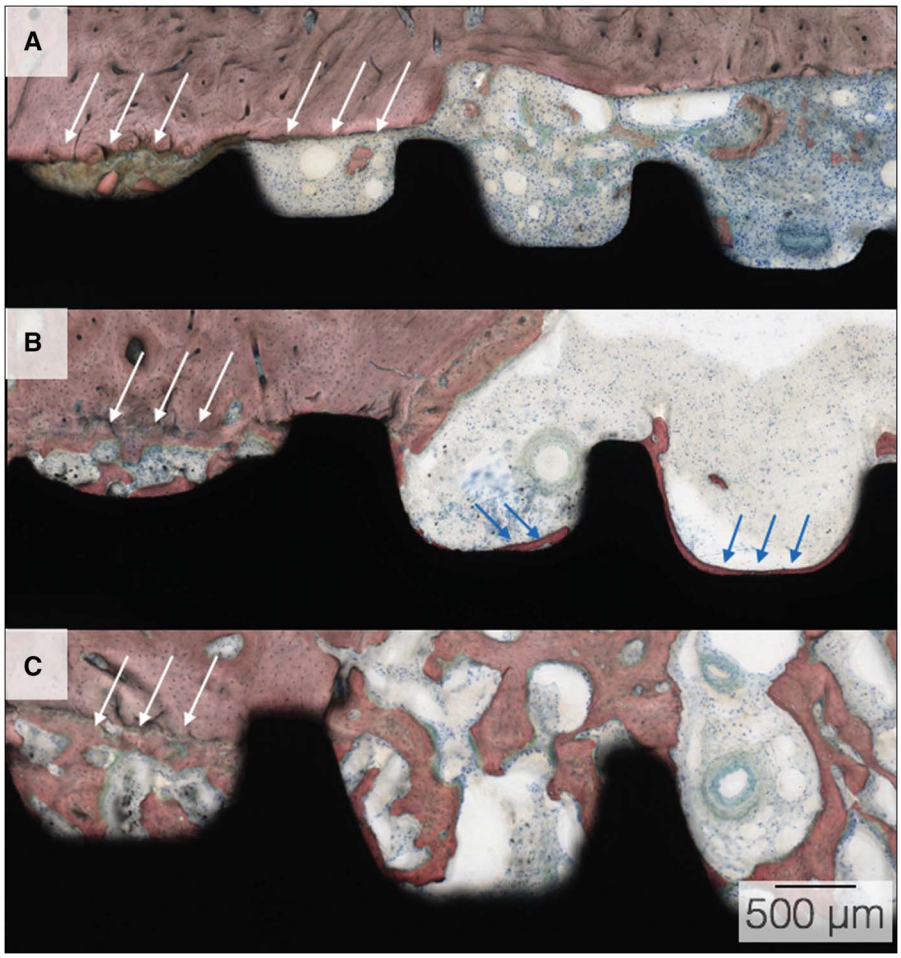

Fig. 5. Representative histologic sections of implants that remained *in vivo* for (**A**) 1 week, (**B**) 3 weeks, and (**C**) 6 weeks. The white arrows depict the healing chamber formation between the instrumented cortical shell and the implant inner diameter, and the blue arrows depict new bone formation in direct contact with the implant surface. Stevenel and Van Gieson staining.

Wetzlar, Germany) with the aid of computer software (Image J; NIH, Maryland).

IFM parameter statistical analysis was performed by one-way analysis of variance (ANOVA). The *in vivo* laboratory model statistical analyses were performed by a mixed-model ANOVA, considering time and implant substrate as independent variables and BIC and BAFO as dependent variables. The statistical unit considered was the number of animal subjects ($n = 18$). All analyses were conducted at $\alpha = 0.05$.

Results

SEM at various magnifications depicted different surface etching patterns for different substrate alloys. The G4 alloy substrate implant presented more uniform etching throughout the structure, whereas the G5 alloy presented less uniform etching pattern characteristic of one of the titanium phases of the biphasic alloy, etching preferentially one relative to the other (Fig. 1). The IFM metric results, on the other hand, revealed that both surfaces are statistically homogeneous for Sa, Sq, and Sdr values (all P values larger than 0.74, Table 1).

Surgical interventions and postoperative period occurred with no complications, and all devices were clinically stable immediately after the euthanasia. No signs of inflammation or infection were observed in the tissue surrounding the implanted devices.

The histomorphometric results (BIC and BAFO) are presented collapsed over the independent variables time *in vivo* and titanium substrate material in Figures 2 and 3, respectively. For BIC, no significant effect of substrate alloy was detected ($P > 0.25$) (Fig. 2, A) while a significant effect of time ($P < 0.001$) (Fig. 3, A) between any given evaluation time *in vivo* was observed. For BAFO, no significant effect of substrate alloy was detected ($P > 0.63$) (Fig. 2, B), and a significant difference was detected between the first time point (1 week) and both subsequent evaluation points (3 and 6 weeks) ($P < 0.001$) (Fig. 3, B). No significant interaction effect between alloy substrate and time was observed for both BIC ($P > 0.83$) and BAFO ($P > 0.95$) (Fig. 4).

Morphological evaluation of the histologic sections demonstrated remarkable similarities between groups at different time points *in vivo*. At 1 week (Fig. 5, A), little new bone formation was observed within the healing connective tissue that was in intimate contact with the implant surface. The formation of a healing chamber between the drilling line and the implant inner diameter bonded by the implant threads was also depicted. This healing chamber formation occurred only at the cortical shell level, and this volume presented connective tissue along with bone chips from the surgical instrumentation. At 3 weeks (Fig. 5, B), new bone formation in direct contact with the implant surface was observed for both groups at all bone regions. At the cortical shell region where a bone chamber was formed, substantial woven bone formation was observed. At the marrow region, a thin layer of bone was observed in direct contact with the implant surface. At 6 weeks (Fig. 5, C), the healing chamber regions were filled with woven bone and an onset of woven bone replacement by lamellar bone was depicted in most samples. At the marrow region, qualitatively higher amounts of bone were observed in proximity and away from the implant surface.

Discussion

Although CP Ti IV and V titanium alloys (Ti-6Al-4V) have been largely

used in both craniofacial and orthopedic applications, no consensus in the literature exists regarding their biocompatible and osseoconductive properties relative to each other. The rationale behind such questioning lies on the potential leaching of the other major atomic components of the Ti-6Al-4V alloy that may be cytotoxic should degradation of this particular alloy occur.[15]

A survey on the topic reveals a sparse and contradictory database where different grades of CP titanium are compared with Ti-6Al-4V. Such studies have been published in the late 90s by a Swedish group where quantitative and qualitative comparisons were made between alloys in a laboratory rabbit model. In both studies, the implants presenting different substrate alloys were manufactured by manual turning; however, in one study, these were used as-machined final surface finish, whereas in the other, a surface texturing technique was used (TiO_2 grit blasting). Even though similar surface roughness profiles were observed for the implants made of both CP and Ti-6Al-4V substrates grit blasted with 2 different TiO_2 particle sizes, higher surface roughness (Sa and Sdr) was observed for the CP relative to the Ti-6Al-4V implants in the as-machined surface finish, logically explained by the difference in alloy mechanical properties. The *in vivo* results obtained for both studies showed comparable histologic osseointegration levels, whereas removal torque values favored the CP alloy relative to the Ti-6Al-4V (38 N·cm compared with 35 N·cm after 1 year, respectively, possibly explained by the differences in the bone mechanical properties surrounding the different implant alloys).[14,16]

Different from the above-described studies, which used the softest of the CP titanium grades, the present investigation compared Ti-6Al-4V with the highest mechanical properties of CP titanium alloy [grade (IV)]. Over the past decade, CP grade IV has gained popularity as an alternative to Ti-6Al-4V as its properties significantly outscore CP titanium from grade III and below.[11] Such recent drive to the utilization of grade IV CP relative to grade II CP has been justified by both presenting the absence of potentially toxic ions in its composition[17–19] and its mechanical properties that allow the fabrication of implant design and dimensions (ie, narrow and short implants) that deviate from standard implants.[20] Thus, this study aimed to histomorphologically/morphometrically compare the initial bone response to grade IV CP titanium and Ti-6Al-4V implants in a beagle dog radius model.

The surface characterization component of this study revealed that although both alloys presented numerically similar surface roughness pattern, their surface texture was morphologically different. Such difference likely occurred because of multiple factors that include the alloy mechanical properties that resulted in different texture before the acid etching procedure along with selective etching of one Ti phase relative to the other on the biphasic Ti-6Al-4V.

The *in vivo* results obtained revealed that from a histologic standpoint, both substrate alloys were equally biocompatible and osseoconductive because no significant differences were observed in either BIC or BAFO when the results were either collapsed over time or when separated into individual groups as a function of time *in vivo*. Although slightly higher BIC and BAFO levels were observed for the G5 implant substrate relative to G4 in most time frames evaluated, these differences were statistically not significant. In addition, evaluation of BIC and BAFO increase over time was proportional for G4 and G5 implants, indicating similar osseointegration rates. Although significant increases were observed for BIC over every single time point when implant groups were collapsed, such significant difference was only detected for BAFO between 1 and 3 weeks *in vivo*, indicating that bone formation around the implants in this study was starting to level out through the onset of woven bone substitution by lamellar bone while surface osseoconductivity was still playing an effect on initial bone healing around both alloys investigated.

Even though several different titanium alloy grades and alloy types are currently used in dentistry and orthopedics with immediate success, their long-term success remains unclear because of the lack of well-designed prospective and retrospective clinical evaluation in the literature. Albeit this study along with a plethora of others that compare the initial response (typically up to approximately 1 year) strongly suggest similar biocompatible and osseoconductive properties between CP, Ti-6Al-4V, and other alternative alloys such as Ti-Zr and Ti-Zr-Nb at tissue histologic level,[12,13,21,22] very few studies have investigated the mechanisms which could possibly result in a clinical advantage when implant in bone biomechanical competence is concerned.

For instance, an exception would be the combination of a histometric and macrobiomechanical investigation by Gottlow et al that presented favorable results to a Ti-17Zr alloy implant relative to grade II CP titanium.[20] A follow-up study that investigated the mechanical property assessment of bone healing around the same specimens from Gottlow et al[23,24] unequivocally demonstrated remarkably similar bone mechanical properties around Ti-17Zr alloy and grade II CP titanium, suggesting similar mineralization rates for the bone forming in proximity of both alloys.

CONCLUSION

Although our histologic results strongly suggest that both alloys present comparable initial osseoconductive and biocompatible properties, it is strongly recommended that *ad hoc* methods such as nanomechanical bone property assessment and gene expression assays are used to further investigate potential similarities in the bone response to different titanium alloys.

DISCLOSURE

The authors claim to have no financial interest. None of the authors were involved either directly or indirectly, in the products or information listed in the article.

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