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Bone tissue response to plasma-nitrided titanium implant surfaces

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ABSTRACT

A current goal of dental implant research is the development of titanium (Ti) surfaces to improve osseointegration. Plasma nitriding treatments generate surfaces that favor osteoblast differentiation, a key event to the process of osteogenesis. Based on this, it is possible to hypothesize that plasma-nitrided Ti implants may positively impact osseointegration. Objective: The aim of this study was to evaluate the *in vivo* bone response to Ti surfaces modified by plasma-nitriding treatments. Material and Methods: Surface treatments consisted of 20% N_2 and 80% H_2, 450°C and 1.5 mbar during 1 h for planar and 3 h for hollow cathode. Untreated surface was used as control. Ten implants of each surface were placed into rabbit tibiae and 6 weeks post-implantation they were harvested for histological and histomorphometric analyses. Results: Bone formation was observed in contact with all implants without statistically significant differences among the evaluated surfaces in terms of bone-to-implant contact, bone area between threads, and bone area within the mirror area. Conclusion: Our results indicate that plasma nitriding treatments generate Ti implants that induce similar bone response to the untreated ones. Thus, as these treatments improve the physico-chemical properties of Ti without affecting its biocompatibility, they could be combined with modifications that favor bone formation in order to develop new implant surfaces.

Keywords: Bone. Dental implants. Plasma gases. Titanium.

INTRODUCTION

Implant rehabilitation is one of the most common treatments performed in Dentistry, with great aesthetics and functional results and high predictability[18]. Despite the success in most cases, the need for good quality osseointegration in challenging clinical situations, as type IV bone[13], has driven the implant research to the development of new titanium (Ti) surfaces[1].

It has been shown that chemical and topographical Ti surface modifications can affect events related to osseointegration[1,26]. Among surface treatments, plasma has been used in orthopedic implants with good results[11]. Plasma nitriding produces an electrical discharge in a gas mixture containing low-pressure nitrogen allowing the formation of nitride instead of oxide layers[4]. It has been shown that plasma nitriding treatments result in an improved surface hardness without affecting Ti biocompatibility[7].

In addition to the conventional plasma technique named planar, Ti surface can be nitrided using a hollow cathode discharge. The use of the hollow cathode method elevates the plasma ion density making the process more effective and generating stable nitride layer, increased surface roughness, and wettability[4]. In a previous study, we have shown that hollow cathode and planar Ti surfaces slightly favor osteoblast differentiation compared with untreated surface[10]. Considering the *in vitro* promising results, we hypothesized that plasma-

nitrided surfaces may enhance osseointegration of Ti implants. Thus, the aim of this study was to evaluate the *in vivo* bone response to Ti surfaces modified by plasma-nitriding treatments.

MATERIAL AND METHODS

Ti implants

Thirty Ti implants (3.75x8.5 cm) with machined surfaces (Conexão, Arujá, SP, Brazil) were used in this study. Ten implants were treated using the hollow cathode technique, 10 using the planar technique, and 10 were kept untreated (control). The treatment conditions were 20% N_2 and 80% H_2, 450°C, 1.5 mbar during 1 h for planar and 3 h for hollow cathode protocol[3,4]. All procedures were carried out in a sealed stainless steel chamber. Prior to implantation, implants were sterilized by gamma radiation.

Surgical procedures

Fifteen male New Zealand white rabbits (3-4 kg) were used in accordance with the research protocols approved by the Committee of Ethics in Animal Research of the School of Dentistry of Ribeirão Preto, University of São Paulo (10.1.161.53.7). The animals were anesthetized using a subcutaneous injection of acepromazine 1 mg/kg (União Química, São Paulo, SP, Brazil), followed by an intramuscular injection of xylazine 5 mg/kg (União Química) and ketamine hydrochloride 25 mg/kg (União Química). After skin preparation, mepivacaine 2% with epinephrine 1:100,000 (DFL, Rio de Janeiro, RJ, Brazil) was used as local anesthesic. An incision was made in the hind leg and the flat surface and the anteromedial area of the tibia was exposed and selected for implant placement (Figure 1A). Surgical site was prepared using drills (Figures 1B-C) and one implant was placed in each tibia (Figures 1D-F) in a randomized way in terms of surface treatment. The implants were sealed with cover screws and the wounds were closed with 3-0 monocryl sutures (Ethicon, São Paulo, SP, Brazil). Postoperatively, all animals received pentabiotic 0.2 ml/kg (Fort Dodge, Campinas, SP, Brazil) as prophylactic antibiotic therapy and Flunixin megumine 1 mg/kg (Shering-Plough, São Paulo, SP, Brazil) as analgesic medication. After 6 weeks, the animals were euthanized with a lethal dose of pentobarbital and the implants were harvested and processed for histological and histomorphometric analyses.

Histological and histomorphometric analyses

Histological and histomorphometric evaluations were done according to the method described elsewhere[16]. The tibia-implant blocks were fixed in 10% formalin buffered with 0.1 M sodium cacodylate, pH 7.3, for 48 h and transferred to a solution of 70% ethanol for 72 h. After dehydration, bone segments were embedded in Hard Grade LR White resin (London Resin Company, London, UK) and sectioned using Exakt Cutting System (Exakt, Norderstedt, Germany). The longitudinal sections obtained were polished and mounted on acrylic slides using Exakt Grinding System (Exakt). The resulting 40 µm thick sections were reduced to a thickness of

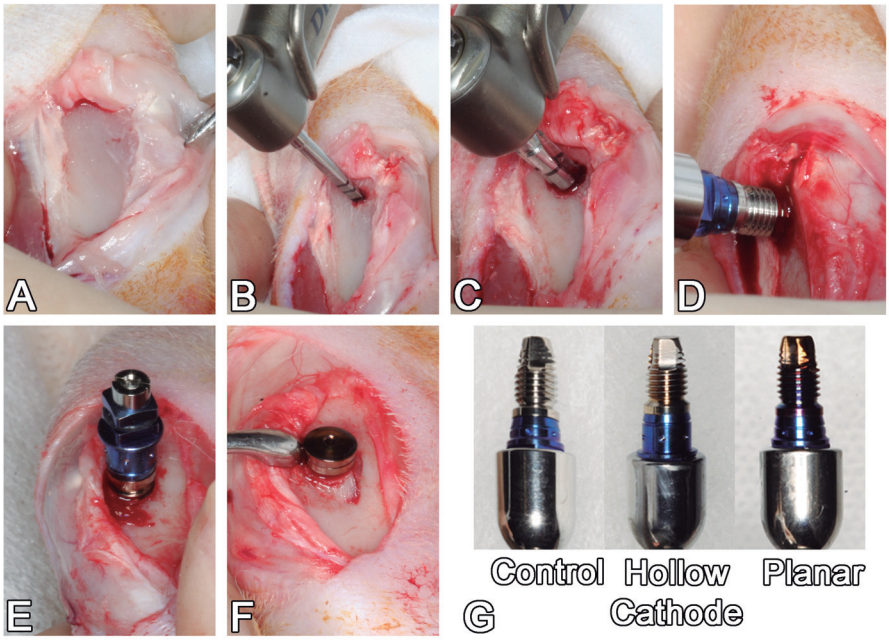

Figure 1- Surgical procedure for implant placement. Total thickness incision and surgical site exposure at the anteromedial region of the tibia (A); drilling the surgical site (B-C) and implant insertion (D-F); View of the implants (G)

20 µm and stained with Stevenel's blue and Alizarin red S. Histological and histomorphometric analyses were carried out by a single examiner based on light microscopy observations using a Leica DMLB light microscope (Leica, Bensheim, Germany) and the ImageJ software, version 1.34 s (NIH, Bethesda, MD, USA). The amount of bone at the bone–implant interface was expressed as bone-to-implant contact (BIC) and, between threads, as bone area between threads (BABT). The amount of bone located outside the threads was determined as bone area within mirror area (BAMA). We previously defined this mirror area as a symmetric area to the trapezoid between two threads, sharing the larger base of the trapezoid[21].

Statistical analysis

Normality of data was determined using the Kolmogorov-Smirnov test. Then, histomorphometric parameters of the three evaluated surfaces (n=10 for each surface) were compared by one-way ANOVA followed by Tukey's test when appropriated and the significance level was set at 0.05.

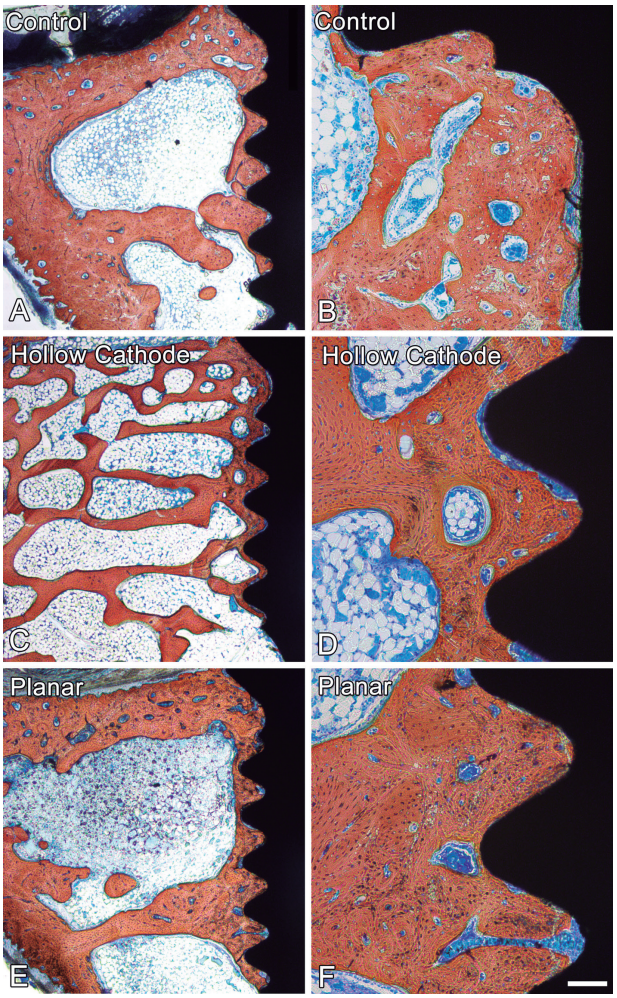

Figure 2- Longitudinal sections of control (A-B); hollow cathode (C-D) and planar (E-F) Ti implant surfaces surrounded by bone and connective tissue, at 6 weeks. Stevenel's blue and Alizarin red S. Scale bar: A, C and E=500 µm and B, D and F=125 µm

RESULTS

Bone formation was observed in close contact with all implants without relevant histological differences among the three evaluated surfaces (Figures 2A, C, and E). Implant surfaces were surrounded by lamellar bone and, at higher magnification, connective tissue was noticed between bone tissue and implant surfaces (Figures 2B, D, and F). The percentage of BIC was 24.5±14.9, 29.8±17.4, and 24.1±13.2 for control, hollow cathode, and planar surfaces, respectively, without statistically significant difference (p=0.737) among them (Figure 3A). The percentage of BABT was

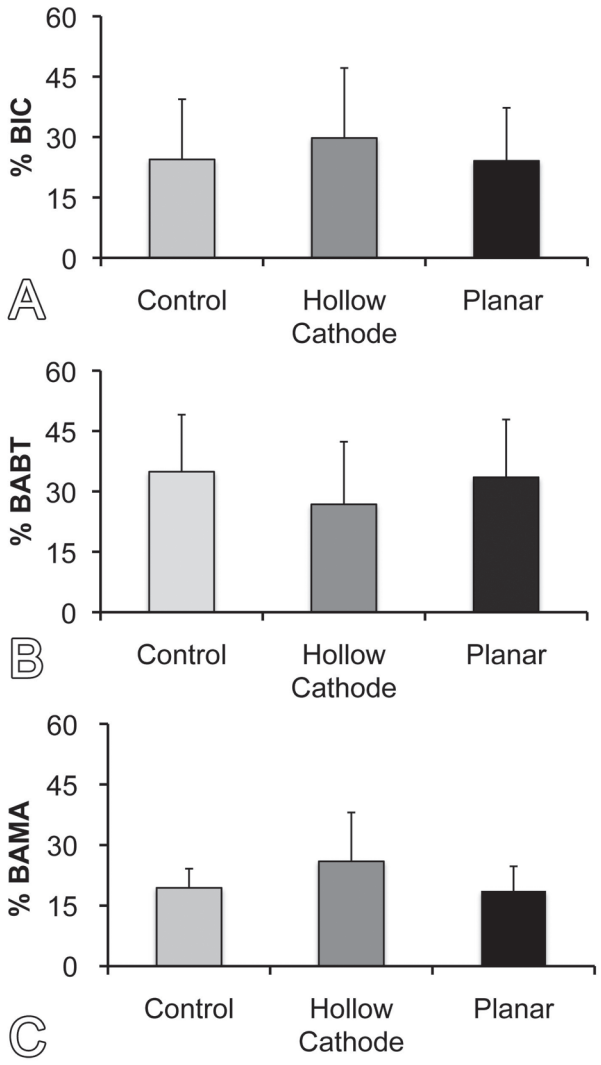

Figure 3- Bone formation around Ti implant surfaces placed into rabbit tibiae at 6 weeks. (A) Bone-to-implant contact (BIC); (B) Mineralized bone area between threads (BABT); (C) Mineralized bone area within mirror area (BAMA). The percentage of BIC, BABT, and BAMA was not affected by surface treatments (p>0.05)

34.8±14.2, 26.8±15.5, and 33.5±14.4 for control, hollow cathode, and planar surfaces, respectively, without statistically significant difference (p=0.445) among them (Figure 3B). The percentage of BAMA was 22.6±4.3, 26.9±9.9, and 22.1±6.2 for control, hollow cathode, and planar surfaces, respectively, without statistically significant difference (p=0.637) among them (Figure 3C).

DISCUSSION

Several Ti surface modifications have been proposed in order to improve the process of implant osseointegration[1,2,6,14,15,17,20]. As plasma-nitrided Ti surfaces favor osteoblast differentiation, here, we have investigated bone tissue response to these surfaces and compared with machined ones. The results showed bone formation in close contact with all implant surfaces without relevant differences in terms of histological and histomorphometric parameters, indicating the lack of effect of plasma nitriding treatments on Ti implant osseointegration.

It has been reported that plasma nitriding treatments affect chemical, topographical, and roughness features[4,10], improving surface hardness without affecting Ti biocompatibility[5,7,22]. Compared with conventional techniques, plasma treatment is inexpensive and environment friendly, needs low temperature and short time treatment and generates a uniform thickness layer[3,23]. In terms of topography and roughness, it was previously observed that plasma-nitrided Ti discs exhibit less homogeneous and rougher discs compared with untreated surfaces, mainly those ones submitted to hollow cathode treatment[10]. Additionally, this treatment results in higher percentage of Ti and contributes to the cleaning of surface as noticed by the reduction of C and O percentage[10].

Distinct treatments generate Ti surfaces with different features, which affect bone cell/tissue response. It has been observed that Ti with nanotopography favors osteoblast differentiation in several culture models[14,17,20]. Also, biological coatings such as bone apatite and type I collagen enhance bone formation in contact with Ti implants[6,25]. Regarding nitriding treatments, previous studies demonstrated that Ti surfaces coated with nitride oxide increase cell growth rate and enhance osteoblast differentiation compared with machined surface[8,10,19]. In addition to *in vitro* studies that are useful to assess the influence of Ti surfaces on the osteoblast behavior in a controlled environment, *in vivo* experiments are of relevance as preclinical models. Thus, we have used an animal model to evaluate bone response to Ti implants and no significant differences in terms of histological and histomorphometric parameters were observed when plasma-nitrided surfaces were compared with untreated one. In agreement with this, it has been shown that, despite modifying surface characteristics, bone formation in close contact with Ti implants is not deeply affected by nitriding treatments[9,15,24]. Bone contact, area, and volume were not affected by nitride Ti produced by powder immersion reaction assisted coating when implanted in rat femora for 8 weeks[24]. Nitrided Ti surfaces produced by glow-discharge plasma treatment had no effect on bone contact and area when implanted in rabbit tibia for 1, 3, and 6 weeks[15]. On the other hand, the increased bone contact with nitrided Ti surface produced by plasma vapor observed 2 weeks post-implantation decreased after 1 and 3 months[9]. As our evaluation was carried out at 6 weeks, a period in which the process of bone formation is completed in this animal model[12], it is possible to suggest that some effect of plama-nitrided Ti implant surfaces on bone formation, if any, could be noticed in early time-points.

CONCLUSION

In conclusion, our results showed that the plasma nitriding treatments used here create Ti implants that elicit similar bone tissue response to the untreated ones. Considering that these treatments improve the physico-chemical properties of Ti without affecting its biocompatibility, the association with modifications generated by either nanotechnology or functionalization with growth factors, which may favor bone formation, should be considered for developing new implant surfaces.

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CONFLICTS OF INTEREST

The authors have no financial interest in any company or any of the products mentioned in this article.

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