

RESEARCH ARTICLE



Osseointegration of implant surfaces in metabolic syndrome and type-2 diabetes mellitus

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Funding information

National Institute of Dental and Craniofacial Research, Grant/Award Number: R21DE023649; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES), Grant/Award Number: CNPq307255/2021-2; FAPESP, Grant/Award Numbers: # 2019/08693-1, BEPE #2021/08018-2, Young Investigators Awards Part I # 2012/19078-7, Young Investigators Awards Part II # 2021/06730-7

Abstract

This *in vivo* study evaluated the bone healing response around endosteal implants with varying surface topography/chemistry in a preclinical, large transitional model induced with metabolic syndrome (MS) and type-2 diabetes mellitus (T2DM). Fifteen Göttingen minipigs were randomly distributed into two groups: (i) control (normal diet, $n = 5$) and (ii) O/MS (cafeteria diet for obesity induction, $n = 10$). Following obesity induction, five minipigs from the obese/metabolic syndrome (O/MS) group were further allocated, randomly, into the third experimental group: (iii) T2DM (cafeteria diet + streptozotocin). Implants with different surface topography/chemistry: (i) dual acid-etched (DAE) and (ii) nano-hydroxyapatite coating over the DAE surface (NANO), were placed into the right ilium of the subjects and allowed to heal for 4 weeks. Histomorphometric evaluation of bone-to-implant contact (%BIC) and bone area fraction occupancy (%BAFO) within implant threads were performed using histomicrographs. Implants with NANO surface presented significantly higher %BIC (~26%) and %BAFO (~35%) relative to implants with DAE surface (%BIC = ~14% and %BAFO = ~28%, $p < .025$). Data as a function of systemic condition presented significantly higher %BIC (~28%) and %BAFO (~42%) in the control group compared with the metabolically compromised groups (O/MS: %BIC = 14.35% and %BAFO = 26.24%, $p < .021$; T2DM: %BIC = 17.91% and %BAFO = 26.12%, $p < .021$) with no significant difference between O/MS and T2DM ($p > .05$). Statistical evaluation considering both factors demonstrated significantly higher %BIC and %BAFO for the NANO surface relative to DAE implant, independent of systemic condition

($p < .05$). The gain increase of %BIC and %BAFO for the NANO compared with DAE was more pronounced in O/MS and T2DM subjects. Osseointegration parameters were significantly reduced in metabolically compromised subjects compared with healthy subjects. Nanostructured hydroxyapatite-coated surfaces improved osseointegration relative to DAE, regardless of systemic condition.

KEYWORDS

dental implants, metabolic diseases, osseointegration

1 | INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies characterized by an abnormal metabolism of carbohydrate, lipid, and protein, resulting in disturbances of insulin secretion and/or cells response to insulin.¹ The fundamental biological concern of DM lies in the alteration of carbohydrate metabolism as the absence or resistance to insulin leads to a deficiency in the uptake or utilization of glucose by cells,^{1,2} increasing blood glucose concentration, and the utilization of fats and proteins as cells fuel source.¹⁻³ Two primary types of DM have been reported: (i) type 1 diabetes mellitus (T1DM) and (ii) type-2 diabetes mellitus (T2DM). T1DM develops due to the destruction of pancreatic beta (β) cells as a result of an autoimmune condition triggered by genetic susceptibility and environmental factors, which induces total absence of insulin secretion (insulin-dependent DM).^{1,3,4} T2DM is caused by a decrease in the sensitivity of target tissues to insulin, which results in an initial hyperinsulinemia that progressively leads to pancreatic beta (β) cell failure due to exhaustion.^{1,3,4}

T2DM is primarily attributed to the establishment of an insulin resistant state that in various circumstances is a progression of the obesity/metabolic syndrome (O/MS) pathophysiology.⁵ First, obesity leads to an increase in the adipose tissue mass, which is stored within and around tissues and organs that ordinarily do not store lipids, as well as an increase in the free fatty acid (FFA) levels in the bloodstream, which is derived from meal and/or abdominal fat lipolysis.^{6,7} Enhanced levels of FFAs may further compromise carbohydrate metabolism due to lipid mitochondrial oxidation at the expense of glucose, a scenario commonly associated with an establishment of a chronic hyperglycemic condition due to a decrease in the glucose uptake and glycogen synthesis and, consequently, hyperinsulinemia.^{1,3,7} A perpetuate hyperglycemic state triggers pathological glycosylation of proteins and formation of toxic metabolites, such as the advanced glycosylation end products (AGEs) that lead to cellular damage and dysfunction, intensifying the impaired glucose metabolism and insulin resistance state.³ In addition, pro-inflammatory cytokines from adipose tissue potentially create a state of chronic systemic inflammation.^{8,9} All of these disturbances of O/MS, associated with genetic predisposition, are major factors for establishing an insulin resistance state, β -cells exhaustion, thus accelerating the onset of T2DM.⁷

Considering the ever increasing prevalence of O/MS and T2DM, which is estimated to affect more than 50% of adult population of the

world by 2050,^{7,10} and the higher level of severe periodontal disease and tooth loss in those subjects,¹¹ a substantial number of patients with metabolic diseases looking for implant-supported rehabilitations can be expected. The success of treatment with dental implants primarily depends on osseointegration, which is the phenomenon where bone directly opposes an implant device without the interposition of soft tissue,¹² and it is highly dependent on bone metabolism.¹³ However, the basic processes of osseointegration, including hemostasis, inflammation, proliferation, and remodeling are negatively affected in metabolically compromised patients. The impairment in the bone healing mechanism has been associated with a reduction of vascular supply due to microangiopathies, dysfunctional cellular activity as a result of the presence of toxic metabolites, and decreased host immune resistance considering the chronic pro-inflammatory systemic state.¹⁴ Previous studies have demonstrated that such disorders affect the rate of bone formation and resorption by decreasing the differentiation of mesenchymal stem cells, reducing the function of osteoblasts, and enhancing osteoclast-based bone resorption.^{4,7,15-18} Also, the pro-inflammatory state and the accumulation of toxic metabolites have shown to affect the collagen structure, compromising organic matrix and, consequently, bone quality.^{4,7,19,20}

It has been well-established that the structural union between the device and living bone might be greatly influenced by implant's surface properties, where numerous chemical/topographic modifications have been proposed to facilitate osseointegration,²¹⁻²⁴ particularly in challenging clinical situations such as low-density bone regions and systemically compromised hosts.²⁵ The current industrial gold standard for implant surface modification encompasses texture modification at the micrometer length scale, that is, grit-blasting/acid etching or dual acid-etching (DAE), which have demonstrated an enhanced blood clot interaction with the device due to an increase in the surface area along with a upregulation of the differentiation of osteogenic cells, resulting in a higher degree/quality of bone formation relative to smooth (as-machined) surfaces.^{26,27} Despite the success obtained by micrometer scale texturing, it has been shown that native tissues arise from a molecular organization with size varying from nano- to micro- and macrometer length scale,^{28,29} supporting the development of implant surfaces that topography hierarchically include nanometer scale within micrometer length scale. Such an approach has already been shown to be effective in allowing interaction between surface and host proteins and cells, positively influencing osseointegration.^{30,31} Moreover, nanostructured calcium

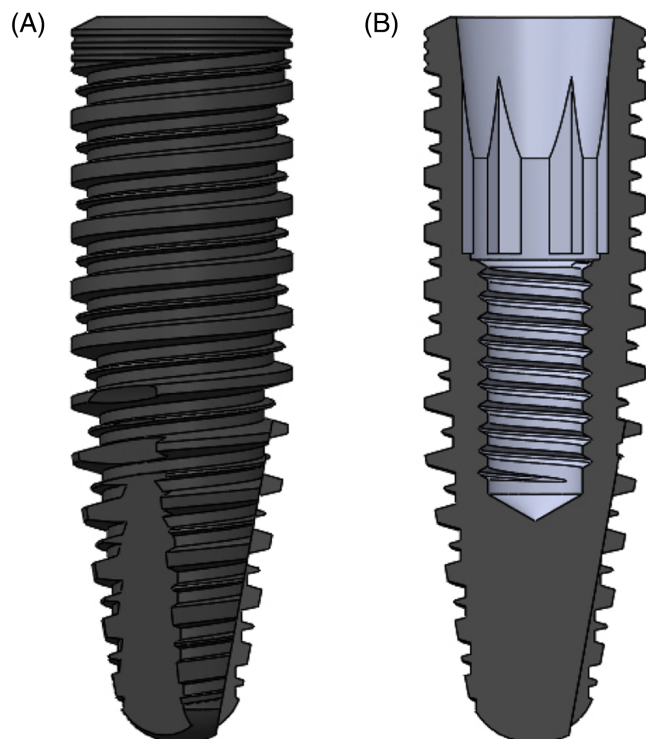


FIGURE 1 (A and B) Implant macrogeometry. Internal conical implants with internal and external trapezoidal threads and a tapered apex, which allow for the formation of healing chambers.

phosphate (CaP) coatings on implant surfaces have shown to enhance bone formation rate and quality, improving bone mechanical properties, at earlier stages relative to conventional surface modifications at micro- and nanometer length scale.^{32,33} CaP coating influence on bone healing response might lie in the association of the positive influence of nanotopography with the inherent CaP osteoconductive properties.³⁴

An understanding of the biological response around implant surface chemistry/texture modifications and their potential benefit to the impaired bone healing in metabolically compromised subjects, O/MS and T2DM, becomes paramount.²⁹ Therefore, the present study evaluated the effect of different implant surface modifications on O/MS and T2DM systemic conditions relative to a healthy control using histological/metric osseointegration parameters. The postulated null hypothesis was that implant surface chemistry/texture modifications would not improve osseointegration histologic parameters in metabolically compromised systemic conditions.

2 | MATERIALS AND METHODS

2.1 | Implants

Commercially pure grade 4 cold worked titanium implants (4.3×10 mm) were selected for the present study (Unitite, S.I.N. Implant system, Sao Paulo, Brazil) ($n = 30$). The implant

macrogeometry consists of internal and external trapezoidal threads and a tapered apex that combined with the surgical instrumentation protocol allows for healing chambers formation, with a blood clot filling the space, and favorable initial stability with lower compression of the bone tissue (Figure 1). Two different types of implant surfaces were investigated: (i) DAE and (ii) 20 nm-coated nano-hydroxyapatite over DAE surface (NANO) (Promimic's HA^{nano} surface, Promimic, Gothenburg, Sweden). The morphology of the implants surface was examined by a field-emission gun scanning electron microscope (FE-SEM, MIRA-TESCAN, Brno-Kohoutovice, Czech Republic) from $1000\times$ to $100,000\times$, operating at an acceleration voltage of 5 kV using a secondary electron detector. The chemical composition of the implants was analyzed by energy dispersive spectroscopy (EDS) (INCA Energy EDS System, Oxford Instruments, Concord, MA, USA; incorporated into a TM-3000 Hitachi micro-scope, Hitachi, Schaumburg, IL, USA).

2.2 | Preclinical in vivo model

The study was performed in accordance with ethical approval from the Institutional Animal Care and Use Committee (Comité d'éthique Anses/ENVA/UPEC #10-0057), as well as adhering to ARRIVE guidelines. The *in vivo* component of the study comprised of a large, highly translational preclinical animal model, Göttingen minipigs (Ellegaard, Dalmose, Denmark), $n = 15$ females, with a mean age of 18 months. The minimum sample size calculated to obtain a statistical test power of 80% and a 5% alpha error within an effect size of 0.80 for a mixed model analysis of the interaction between systemic condition and implant surface was 27 implants, and an extra increase by 10% was planned to account for any potential losses, totaling 30 implants. Given that both implants were nested in the same animal, five animals per systemic condition would be necessary (G*Power 3.1, HHU University, Germany).

2.3 | Establishment of metabolic syndrome and type-2 diabetes mellitus in Göttingen minipig models

Minipigs were randomly distributed by means of a computer-generated randomization list into three groups: (i) control (normal diet), (ii) O/MS (cafeteria diet for obesity induction) and (iii) T2DM (cafeteria diet for obesity induction + streptozotocin for T2DM induction) ($n = 5$ /group). Although a low-fat diet was provided to the healthy control group, the metabolically impaired systemic conditions (O/MS and T2DM) groups were fed with a high saturated and hydrogenated fat/cholesterol/sugar diet.³⁵ The animals were fed twice a day with a combination of either standard diet (SDS—Standard Diet Service, UK# 801586) or cafeteria diet (RDS—Research Diet Services NL), all with the same amount of food by weight.

Three distinct phases were established for animal with weight gain diet protocols: (i) conversion, (ii) growth, and (iii) maintenance. For obesity induction, O/MS and T2DM minipigs were subjected to

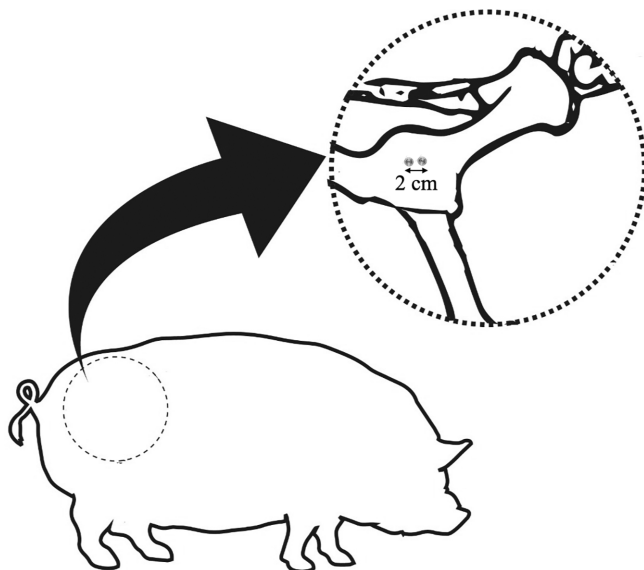


FIGURE 2 Schematic of the implant placement site and distribution. Implants with different surface treatment, NANO and DAE, were installed in the right ilium of the animals in an interpolated distribution (2 cm apart in a mesial-distal fashion), so that the same animal received both implant surface types.

the RDS cafeteria diet for a period of 4 weeks, conversion phase, in which a 25% decrease in the normal diet per week, with a total amount of 500 g of food per meal. Thereafter, O/MS and T2DM minipigs remained at 100% RDS cafeteria diet for approximately 8 months, which is known as growth phase, and were fed ad libitum. Once the minipigs reached the desired body weight (approximately 50% increase relative to their original weight), the cafeteria diet was halved and combined with the SDS standard diet to maintain animal's weight, which is the beginning of the maintenance phase. Control animals were fed with the SDS standard diet and water throughout the experiment.

After obesity induction phase, selected animals received a slow injection using a sterile filter of β -cell cytotoxin streptozotocin solution (STZ) (Enzo Life Sciences, Raamsdonksveer, the Netherlands) following overnight fasting for two consecutive days (20 mg/kg in 0.1 mol/L Na-citrate, pH 4.5), as previously described³⁶ to achieve T2DM subjects. Free access to food following STZ injection was allowed during the daytime and after the second day of injection. To prevent hypoglycemia, the animals were fed with 25 g glucose at the end of each of the first 2 days of STZ treatment to offset insulin release from β -cells. The induction of O/MS and T2DM onset through this proposed methodology was previously validated.²⁵ In the current study, animals were monitored for characterization of metabolically compromised models relative to healthy controls. The following criteria: animal weight and blood analysis, including glucose, cholesterol, triglycerides, and plasma cortisol levels, were used to control the induction of O/MS and T2DM.

Implants with different surface treatment, DAE and NANO ($n = 15$ /surface treatment), were installed in the right ilium in an

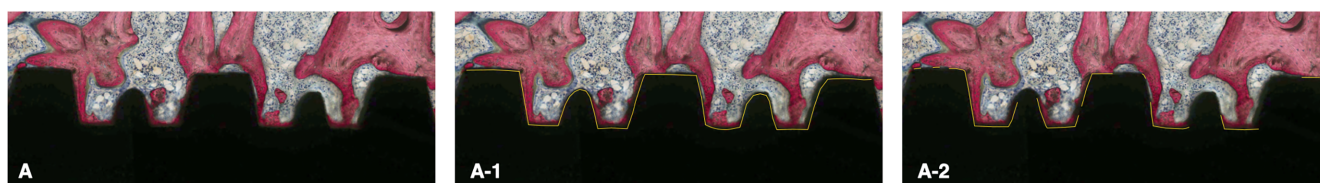
interpolated distribution (through the lateral cortex down to the bone surface, 2 cm apart in a mesial-distal fashion) to minimize bias from different implantation sites, so that the same animal received both implant surface types (Figure 2). The surgeon was blinded for implant type since they present same macrogeometry and osteotomy/ placement protocol; however the assessor in the operating room was aware of the group assignment, being the responsible for providing the implant for the surgeon. The surgical site was shaved and prepared with iodine solution before surgery. Anesthesia was induced with etamine hydrochloride 50 mg, Ketalar® 50 mg/mL (Pfizer, New York, NY, USA), Midazolam 15 mg, and Dormicum® 5 mg/mL (Roche, Basel, Switzerland). ECG, SpO₂, and tidal CO₂ were used to monitor animals. A blanket with circulating hot water was used to maintain body temperature. For implant placement, an incision was first made in the anteroposterior direction over the ilium, followed by dissections of fat tissue, and muscular tissue with blunt dissections and the application of a periosteal elevator. The implant osteotomy followed the drills sequence recommended by the manufacturer under abundant saline irrigation at 1100 rpm. Once the implants were placed, the soft tissue was closed in layers with nylon 2-0 for skin and vicryl 2-0 for muscle. The animals were maintained in animal care facility and received antibiotic (Benzyl Penicilin Benzatine 20,000UI/kg) and anti-inflammatory (Ketoprofen 1% 1 mL/ 5 kg) medication to control the pain and infection. After recovery, the animals were fed with food and water ad libitum. Then, the animals were sacrificed 4 weeks after surgery by anesthesia overdose and samples were retrieved by sharp dissection.

2.4 | Histologic preparation and histomorphometric analysis

The retrieved samples were stored in 70% ethanol for 24 h and subsequently washed under running water for an additional 24-h immersion in 70% ethanol. For histologic processing, the samples were subjected to a progressive dehydration protocol through 70%, 90%, and 100% ethanol solutions; then, embedded in a methacrylate-based resin according to the manufacturer's instructions (Technovit 9100, Heraeus Kulzer GmbH, Wehrheim, Germany). The embedded samples were sectioned along the implant long axis using a precision diamond saw (Isomet 2000, Buehler, Lake Bluff, IL) into thin slices (~250–300 μ m), and the most central slices were glued to acrylic plates with acrylate-based cement. The slides were prepared for histomorphometric analysis by grinding using a series of 400–2400 grit SiC abrasive papers and polishing under water irrigation until a slice final thickness of ~100 μ m in a grinding/polishing machine (Metaserv 3000, Buehler, Lake Bluff, IL, USA). Subsequently, the samples were stained with Stevenel's Blue and Van Giesons's Picro Fuschin stains, then an automated slide scanning system and specialized computer software were used to scan the slides (Aperio Technologies, Vista, CA, USA).

Qualitative and quantitative histological evaluation were conducted with the percentage of bone-to-implant contact (%)

$$\%BIC = \text{Bone Perimeter (A-2)} \times 100 / \text{Implant Perimeter (A-1)}$$



$$\%BAFO = \text{Bone area (A-5)} \times 100 / \text{Thread Area (A-4)}$$

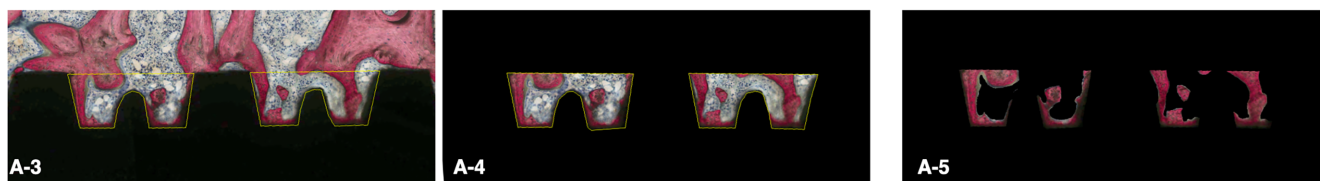


FIGURE 3 Percentage of bone-to-implant contact (%BIC) and percentage bone area fraction occupancy (%BAFO) illustration, where %BIC was determined by the ratio between the perimeter of the implant surface with new bone (depicted in A-2) and perimeter of the entire implant (depicted in A-1). %BAFO was determined by the ratio between the bone fraction area within the threads (depicted in A-4) and the total area of the threads (depicted in A-4).

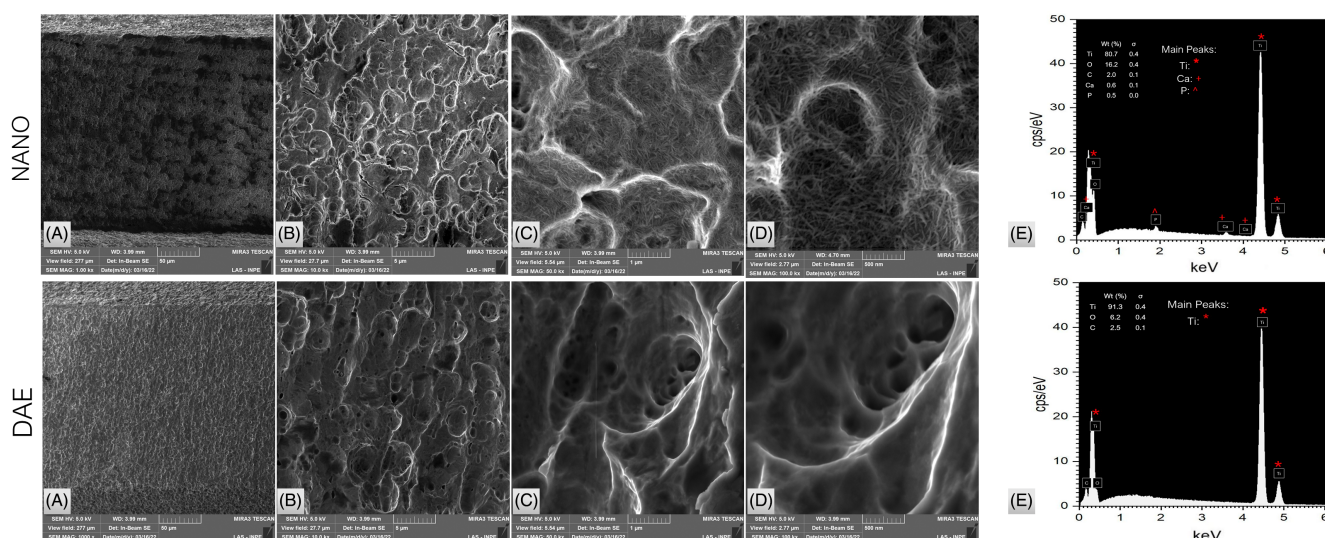


FIGURE 4 (AE). Scanning electron microscopy micrographs at (A) 100 $\times$, (B) 1000 $\times$, (C) 5000 $\times$, and (D) 10,000 $\times$. (A–C) Surface shows a homogeneous pattern of micropores, with larger craters embracing smaller grooves for both implants, which is usually consistent with patterns observed in acid etched dual acid-etched (DAE) implants. (D) The regular homogeneously distributed nanoporosity can be observed for the NANO implant. (E) Chemical element analysis depicts the presence of Ca and P on the surface of the NANO implants.

BIC) and bone area fraction occupancy (%BAFO) within the implant threads quantified by means of a computer software and a single blinded operator performed all the analyses (Image J; National Institutes of Health, Bethesda, MD Leica Application Suite, Leica Microsystems).²⁷ %BIC was determined by the ratio between the perimeter of the implant surface with new bone and perimeter of the entire implant. %BAFO was determined by the ratio between the bone area of bone fraction within the threads and the total area of the threads (Figure 3).

2.5 | Statistical analysis

Systemic condition and implant surface treatment were the independent variables of the study. Although %BIC and %BAFO were the dependent primary variables; animal weight and blood analysis parameters were the dependent secondary variables. Data were collected and statistically evaluated through linear mixed models with fixed factors of systemic condition (control, O/MS, and T2DM) and implant surface treatment (DAE and NANO) following a post hoc comparison of the means using Tukey test. The data were presented in figures

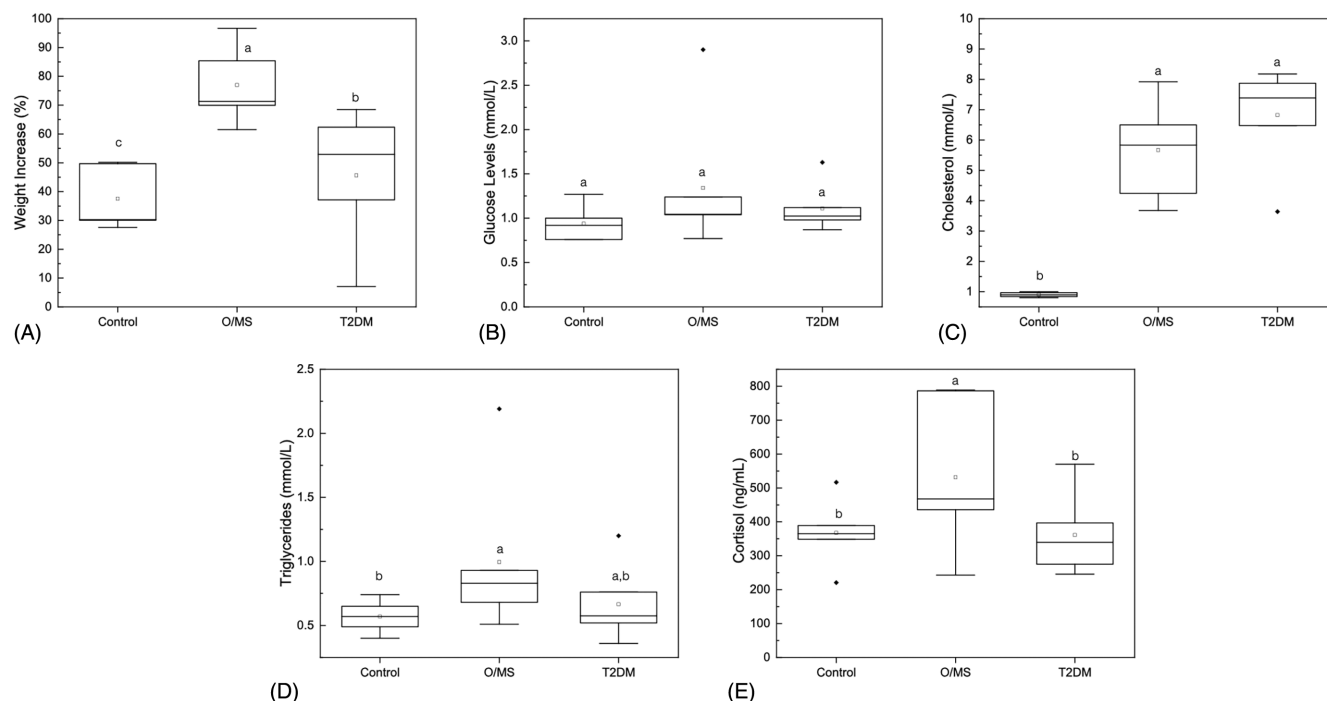


FIGURE 5 (A-E). Parameters used to characterize and validate disease induction. (A) Weight increase. (B) Glucose level. (C) Cholesterol. (D) Triglycerides. (E) Cortisol. Identical letters indicate no significant difference among groups. '□' indicates the mean and '♦' indicates an outlier. O/MS, obesity/metabolic syndrome; T2DM, type-2 diabetes mellitus.

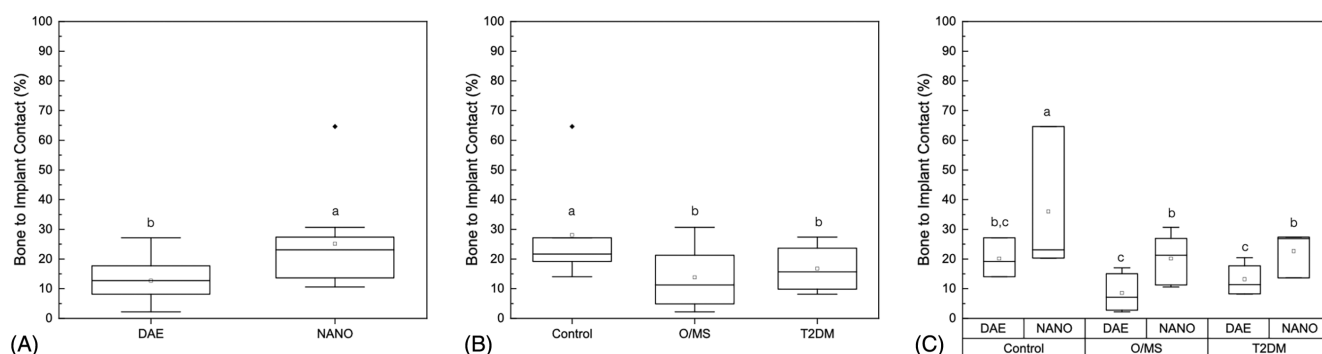


FIGURE 6 (A-C). Percentage of bone to implant contact. (A) Regarding to surface treatment, NANO coated implants depicted higher levels of bone to implant contact than dual acid-etched (DAE). (B) Concerning to systemic condition, control group presented significant more percentage of bone to implant contact when compared with obesity/metabolic syndrome (O/MS) and type-2 diabetes mellitus (T2DM); without significant difference between them. (C) All NANO coated groups demonstrated higher percentage of bone to implant contact when compared with their DAE counterparts. Identical letters indicate no significant difference among groups. '□' indicates the mean and '♦' indicates an outlier.

with means, medians, and respective interquartile ranges with $p < .05$ considered statistically significant. All analyses were accomplished using SPSS (IBM SPSS 23, IBM Corp., Armonk, NY).

3 | RESULTS

Scanning electron microscopy micrographs demonstrated a homogeneous pattern of micropores on both implants' surfaces, DAE and nano-hydroxyapatite over DAE (NANO), with the presence of larger and smaller grooves, consistent with the process of acid etching

(Figure 4A-C). High magnification micrographs depict regular and homogeneously distributed nanoscale surface texture for the NANO implants (Figure 4D). The survey spectra of the samples obtained in the EDS analysis showed major peaks in correspondence to the wavelengths of titanium, oxygen, and carbon for either DAE or NANO implants; however, NANO implants also exhibited peaks in correspondence to the wavelengths of Ca and P on the surface, which is consistent with the hydroxyapatite coating process (Figure 4E).

Preclinical experimentation, surgical interventions and follow-up visit data have demonstrated no complications regarding procedures, postoperative infections and/or other clinical concerns. No implants

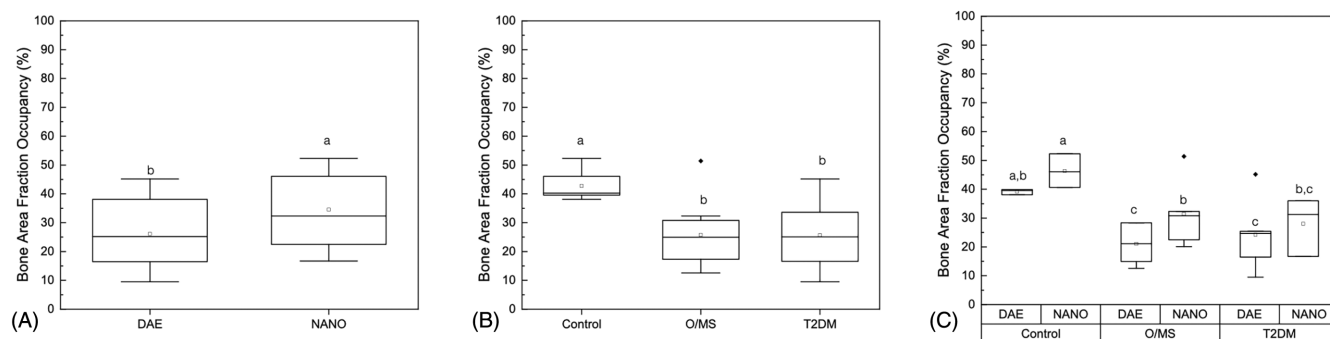


FIGURE 7 (A–C). Percentage of bone area fraction occupancy. (A). Regarding to surface treatment, NANO coated implants depicted higher levels of bone area fraction occupancy within healing chambers. (B) Control group presented significant more percentage of bone area fraction occupancy when compared with obesity/metabolic syndrome (O/MS) and type-2 diabetes mellitus (T2DM) conditions, without significant difference between them. (C) Higher percentage of bone area fraction occupancy for control groups when compared with O/MS and T2DM. All NANO coated groups demonstrated higher percentage of bone area fraction occupancy when compared with their dual acid-etched (DAE) counterparts, with significant difference for O/MS group. Identical letters indicate no significant difference among groups. '□' indicates the mean and '♦' indicates an outlier.

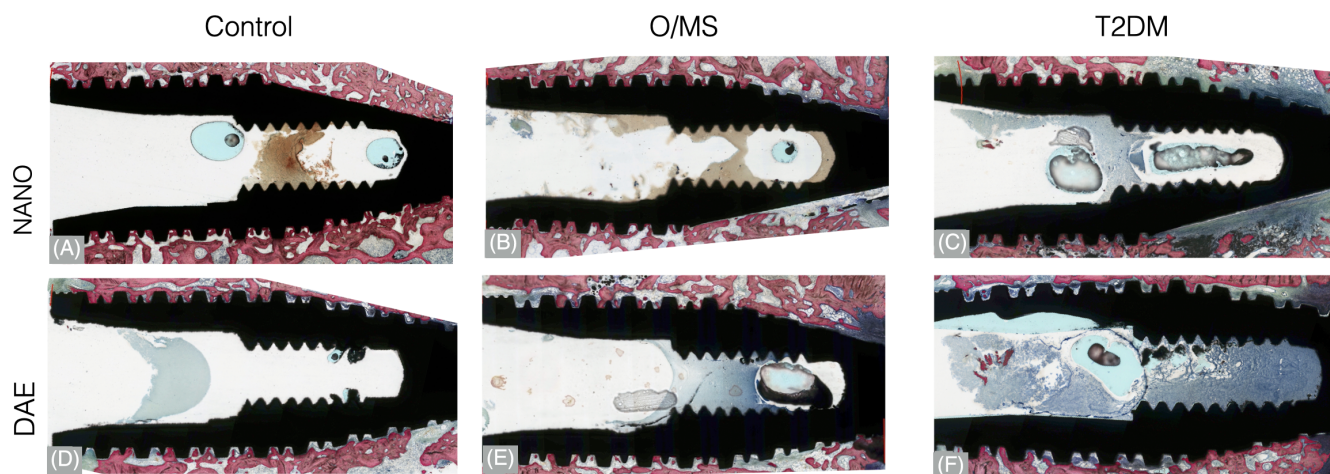


FIGURE 8 Histological sections of control (A and D), obesity/metabolic syndrome (O/MS) (B and E), and type-2 diabetes mellitus (T2DM) groups (C and F) for NANO coated (A, B, and C) and dual acid-etched (DAE) treated (D, E, and F) implant surface treatments. More pronounced bone formation in direct contact and in proximity with the implant is evidenced for NANO surface treatment, regardless group. Also, control group presented higher amount of woven bone when compared with O/MS and T2DM systemic conditions.

were excluded from the study due to their clinical stability immediately after euthanasia, which was confirmed by histologic evaluation.

Comparatively, the weight increase for both groups of pigs receiving the cafeteria diet O/MS and T2DM was significantly higher than healthy (control) pigs ($p < .030$) (Figure 5A). O/MS and T2DM group presented average blood glucose levels approximately 1.4 and 1.3 times greater than control group, respectively, though it was not statistically significant different ($p > .072$) (Figure 5B). Similarly, cholesterol, triglycerides, and cortisol levels of metabolically compromised groups were significantly higher relative to the healthy control group ($p < .001$), except for T2DM and control pairwise comparisons of triglycerides and cortisol levels ($p > .681$) (Figure 5C–E).

%BIC and %BAFO as a function of surface treatment yielded significant differences between DAE and NANO groups after 4 weeks in vivo, with the hydroxyapatite coated NANO surface outperforming

DAE ($p < .025$) (Figures 6A and 7A). Evaluating as a function of systemic condition (data collapsed over surface treatment), demonstrated higher %BIC and %BAFO in the control group compared with O/MS and T2DM ($p < .021$). No significant difference was observed between O/MS and T2DM ($p > .50$) (Figures 6B and 7B).

Statistical evaluation considering both factors, systemic condition and surface treatment, indicated higher %BIC and %BAFO for the NANO control group when compared with O/MS and T2DM ($p < .027$) (Figures 6C and 7C). One exception was the %BIC for DAE group, which yielded a higher mean value and was not statistically significant. NANO coated implants improved %BIC and %BAFO for all systemic conditions compared with their DAE counterparts and the gain increase in osseointegration indicators due to the NANO surface relative to DAE was more pronounced in the metabolically compromised groups. Additionally, O/MS and T2DM NANO groups

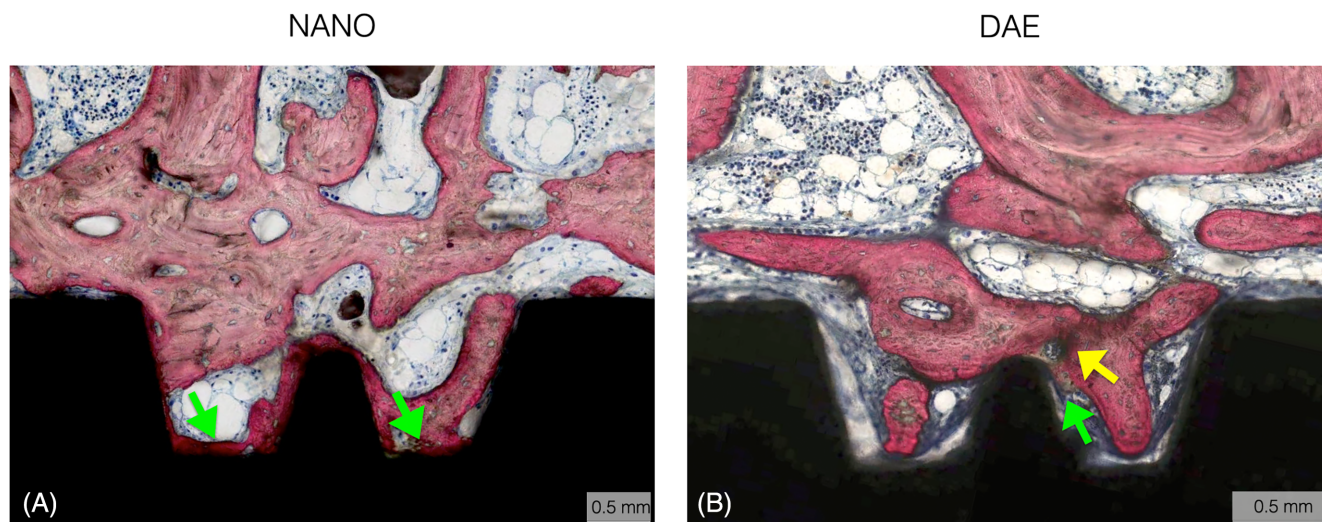


FIGURE 9 Histological micrographs of control group healing chambers for NANO coated (A) and dual acid-etched (DAE) treated (B) surfaces. The green arrows depicted new bone formation in direct contact and in proximity with the implant surface, which was more pronounced for NANO surface. Bone remodeling units were observed (yellow arrow) replacing previously formed bone.

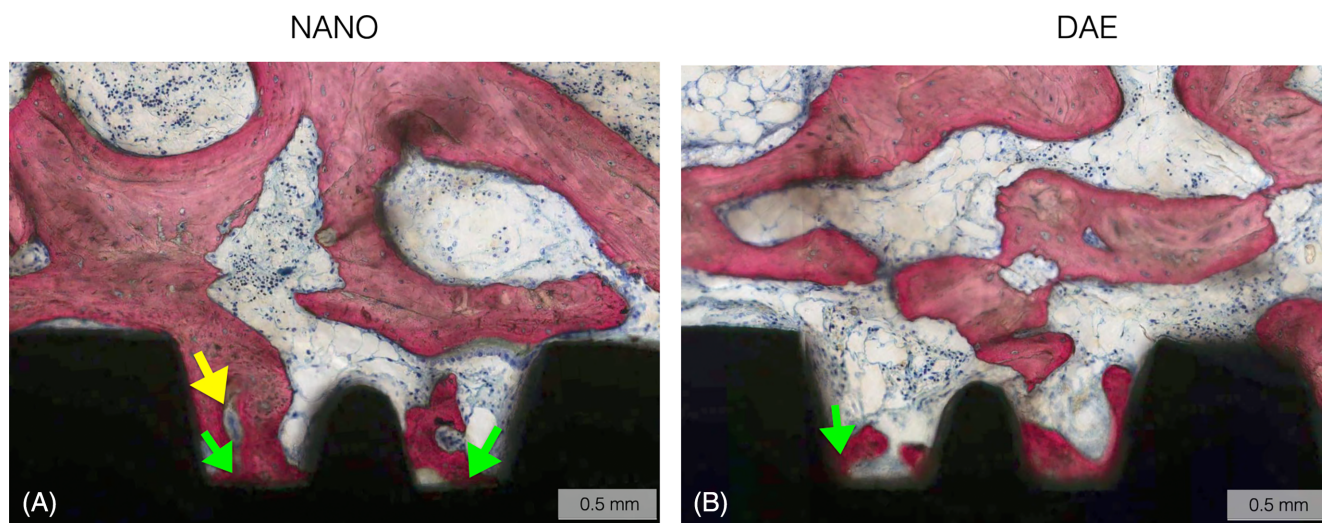


FIGURE 10 Histological micrographs of obesity/metabolic syndrome group healing chambers for NANO coated (A) and dual acid-etched (DAE) (B) treated surfaces. The green arrows depicted new bone formation in direct contact and in proximity with the implant surface, which was more pronounced for NANO surface when compared with DAE. Bone remodeling units were observed (yellow arrow) replacing previously formed bone.

demonstrated comparable levels of %BIC and %BAFO to control DAE, reflecting a favorable effect of nano-hydroxyapatite coating on bone healing improvement in metabolically compromised systemic conditions (Figures 6C and 7C).

Representative histological micrographs of all groups are presented in Figure 8. The histological micrographs indicated successful osseointegration for all implants. Independent of implant surface treatment, osseointegration occurred through interfacial remodeling at the thread tip as a result of compression, and through an intramembranous-like process within the healing chambers that were created due to the mismatch between final osteotomy diameter and implant inner diameter. Such space, according to the manufacturer,

was bound at the outer diameter of the smaller thread component of the implant and the implant inner diameter. Histomorphologic evaluation demonstrated that, independent of experimental group, the healing chambers were filled with woven bone for all groups. Nonetheless, more pronounced bone formation along with the implant surface was observed for hydroxyapatite-coated NANO implant surfaces when compared with DAE, regardless of systemic condition. Also, the control healthy group presented higher amounts of new bone relative to O/MS and T2DM. High magnification optical micrographs of NANO and DAE implant healing chambers for control, O/MS, and T2DM groups are presented in Figures 9–11. The representative micrographs depict woven bone in intimate contact and in

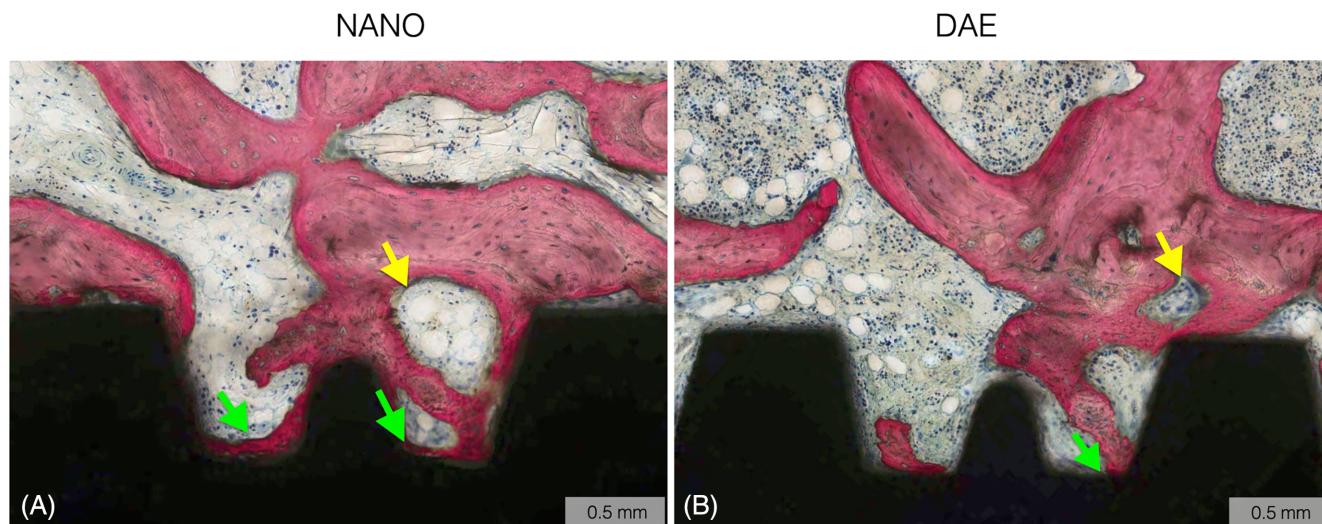


FIGURE 11 Histological micrographs of obesity/metabolic syndrome (type-2 diabetes mellitus) group healing chambers for NANO coated (A) and dual acid-etched (DAE) (B) treated surfaces. The green arrows depicted new bone formation in direct contact and in proximity with the implant surface, which was more pronounced for NANO surface when compared with DAE. Bone remodeling units were observed (yellow arrow) replacing previously formed bone.

proximity with the implant surface along, especially for the NANO implants, with bone remodeling units for all systemic conditions and implant surface treatment.

4 | DISCUSSION

Although implant rehabilitation is a reliable treatment modality, it primarily relies on a successful interlocking between newly-formed bone and implant surface, named as osseointegration.¹² This is a complex and dynamic process, which is based on a healthy bone metabolism to support wound healing and %BIC establishment.^{4,13} Osseointegration is known to be impaired in metabolically compromised systemic conditions, such as O/MS and T2DM.^{4,19,20,37–39} In order to improve the rate and extent of bone healing around metallic implants in such challenging scenarios, implant surface topography/chemistry modifications have been suggested.^{26,29,40} Therefore, the current study aimed to evaluate the effect of implant surface modifications in metabolically compromised subjects, O/MS and T2DM, relative to healthy controls, using histologic/metric osseointegration indicators. Overall, %BIC and %BAFO were significantly higher for nano-hydroxyapatite coated (NANO) implants relative to DAE implants independent on the systemic condition, with more pronounced increase in the bone formation for O/MS and T2DM groups. Hence, the postulated null hypothesis that implant surface chemistry/texture modifications would not improve osseointegration histologic parameters in metabolically compromised systemic conditions has been rejected.

Concerning systemic condition, the control (healthy) group presented relatively higher %BIC and %BAFO compared with the O/MS and T2DM subjects. Histological micrographs also suggested a more pronounced bone formation along and in proximity with the implant surface for the healthy control group relative to O/MS and T2DM

groups. The rationale behind this result lies in the fact that metabolically compromised systemic conditions are associated with a reduction in the vascular supply, accumulation of AGEs, and a perpetuated pro-inflammatory state that negatively affect osseointegration establishment and maintenance.^{4,7,15–18} Alterations in the organic and bone structures, such as impaired collagen arrangement and imbalance between osteoblast and osteoclast activity, also result in an overall deterioration of the structure, quality, and stiffness of bone tissue.^{4,19,20} Consequently, the healing process has been shown to be delayed around implants placed in metabolically compromised subjects.³⁹

To the best of our knowledge, there is a paucity in literature regarding the effect of O/MS on osseointegration.²⁵ However, previous findings have demonstrated decreased levels of bone formation around implants placed in individuals with T2DM relative to healthy control.^{4,41,42} In the present study, no significant differences were observed when O/MS and T2DM groups were compared, irrespective of histological parameter. The parallel pathophysiology between O/MS and T2DM, including the presence of toxic metabolites and systemic pro-inflammatory systemic condition, can be associated with the absence of significant difference between these metabolic diseases for all parameters evaluated.⁵ Some clinical concerns arise when considering the comparable osseointegration impairment between O/MS and T2DM, suggesting that clinicians should take into consideration O/MS patients as high-risk similar to T2DM. Moreover, elucidation of the mechanisms that link O/MS and bone healing process would further help the understanding of the osseointegration impairment, as well as the development of strategies for new therapeutic approaches.

Targeting to improve the osseointegration process, implant surface chemistry/texture modifications have been proposed.^{26,27,29,43–45} In the present study, the morphological and chemical element

analyses of the implants' surface revealed a microporous texture, with larger grooves illustrating an arrangement of smaller grooves for both implants surface that might increase surface area; however, at higher magnifications, a hierarchically structured nanotopography with the presence of Ca and P elements could be observed for the NANO group. Such a more complex and biologically active structure resulted in a higher %BIC and %BAFO for the NANO group relative to the DAE group, outcome also validated with the histologic micrographs. The development of complex surfaces with chemistry/topography modifications, such as bioactive nano-hydroxyapatite coated implants (NANO), have provided implant systems with a more favorable surface arrangement, mimicking natural tissue organization, to facilitate the interaction with biomolecules and favor cell communication during osseointegration process.^{30,31,46} Previous studies comparing the use of NANO and DAE surfaces have also shown an improved healing process for the former with respect to histological parameters.^{32,47,48}

The favorable results associated with complex implant surfaces in challenging clinical scenarios, such as low-density bone sites, encouraged their use in compromised systemic conditions that potentially affect bone metabolism and osseointegration.⁴⁸ In the present study, comparisons of NANO to DAE implants in O/MS and T2DM relative to healthy control demonstrated improved %BIC and %BAFO for NANO implants compared with their DAE counterparts, and the gain increase in bone formation for the NANO surface relative to DAE was more pronounced in the metabolically compromised subjects relative to healthy controls. Additionally, O/MS and T2DM NANO groups demonstrated comparable levels of %BIC and %BAFO to healthy control DAE groups, which reflects the potential effect of nano-hydroxyapatite coating on bone healing improvement in compromised health conditions. Previous literature findings have indicated that NANO complex surfaces outperformed microtopography surfaces, including DAE, in metabolically compromised subjects.^{49–51} Consequently, the current results and literature findings support the premise of using of bioactive nanometer length scale surface modifications as a potential compensation for the negative influence of O/MS and T2DM health conditions, warranting further investigations.

For healthy individuals, human retrieval histologic studies have exhibited approximately 40–60% %BIC for implants with a DAE surface placed in the mandible after an osseointegration period of approximately 4 weeks,^{52,53} though others have demonstrated approximately 30% %BIC in the same clinical scenario.⁵⁴ For more complex surfaces, such as the hydrophilic DAE (SLActive®, Straumann), the %BIC has also been estimated to be approximately 48% after 4 weeks.⁵⁴ Considering individuals with O/MS, retrieved mandible implants have exhibited approximately 33% BIC after a healing period of up to 8 weeks.⁵⁵ Such data corroborate with the %BIC estimated in a Göttingen minipig preclinical animal model, in which approximately 50% BIC for healthy subjects and up to 38% %BIC for O/MS and T2DM subjects have been measured after 4 weeks *in vivo* for implants placed in the mandible. On the other hand, for implants placed in the maxilla, especially in the posterior region that is mainly composed of trabecular bone, a lower %BIC relative to the mandible has been reported in human retrieval studies at early healing

periods.⁵⁶ Such findings have indicated a %BIC of approximately 21%–35% for posterior maxilla after a healing period of up to 8 weeks,^{57–59} with a upregulation in the rate of bone formation for more complex implant surfaces compared with DAE surfaces.⁶⁰ Previous studies using swine preclinical models have shown a %BIC of up to 24% after 4 weeks *in vivo* for implants with a DAE surface,^{61,62} with more complex hydrophilic surfaces also outperforming DAE surfaces at early healing periods.⁶³ For implants placed in the ilium using different animal models, which presents predominantly trabecular bone and resembles the posterior region of the maxilla, the %BIC was also approximately 28% at early healing periods for implants with similar macrogeometry and healing pattern herein investigated,^{64,65} where a 10% increase in the %BIC was observed for implants with a NANO structured surface, 38% BIC.⁶⁴ Such results corroborate with the findings presented in the current study, where healthy Göttingen minipigs presented a %BIC of approximately 20% and 35% for implants placed in the ilium with DAE and NANO surfaces, respectively. It is also worthy to mention that despite the expected decrease in %BIC for metabolically compromised subjects, as above discussed, implants with NANO surface increased the %BIC to similar levels of DAE implants placed in healthy subjects, 20% BIC, which is also similar to levels reported in human retrieval studies in the posterior maxilla.^{57–59} Therefore, the rate of bone formation either in the mandible or in the maxilla in preclinical animal models along with other bone areas that present similar bone architecture was comparable to the percentage reported in the human retrieval studies, supporting preclinical studies as a predictable method to evaluate bone healing response to implantable devices. The small disparities between studies may lie in methodological differences, including surgical site, implant macrogeometry, surgical procedure, and osteotomy dimensions, which might affect healing kinetics and, consequently, the amount of bone formation on the implant surface.^{66–68}

Moreover, several methods have been published in order to induce O/MS and T2DM in animals,⁴ however, the choice of a suitable model is based on its ability to mimic the human pathogenesis and expected results.⁶⁹ A substantial part of the current knowledge about the effect of DM on osseointegration is based on small animal models investigating T1DM induced through injections of either STZ or alloxan; however, this procedure destroys pancreatic β -cells resulting in profound hyperglycemia.¹ Given that T2DM accounts up to 90% of diabetes cases and its pathophysiology is directly related to the progression of O/MS associated with genetic factors,^{1,3,4} the development of a model that indeed reflects metabolic changes compromising human body is required to support translational relevance of researches results. Therefore, the present study used the Göttingen minipig model that has been well recognized as a remarkable model in biomedical research due to its anatomical, physiological, and metabolic similarities to the human organism.^{25,70} Minipigs are easy to handle in a laboratory setting compared to other large animal species with an final adult weight of approximately 50–80 kg, depending on the systemic condition.^{25,70} The animals with metabolically impaired systemic conditions of the present study, O/MS and T2DM, underwent a validated high saturated and hydrogenated fats/

cholesterol/sugar diet for an extended period, to gain weight until the O/MS condition was developed.²⁵ After, T2DM animals were administered a low dose of STZ injection to enable a less time-consuming conversion of a prediabetic condition into a frank T2DM. This procedure reduces the insulin secretion of the β -cells, reflecting initially insulin resistance and compensatory hyperinsulinemia followed by a decreased insulin secretion.²⁵ Consequently, animals expressed a human-like disease phenotype, as previously validated²⁵ and characterized by the results obtained for weight increase and blood analyses, as well as confirmed by the histologic/metric results, in which both metabolically compromised systemic conditions negatively influenced bone healing around metallic implants. Although preclinical animal models are a predictable method to investigate the bone healing response around dental implants, there will always be pathophysiological differences that might affect clinical translation of data.⁷¹ Hence, clinical studies are also paramount to investigate the performance of implantable devices in healthy and metabolically compromised systemic conditions, especially to evaluate technical and biological complications.

5 | CONCLUSIONS

Metabolic syndrome (O/MS) and T2DM negatively affect bone healing around metallic implants; however, implant surface modifications, mainly including nanostructured topography and chemistry alterations, have improved histological parameters of osseointegration in such pro-inflammatory systemic conditions.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (NIH)/National Institute of Dental and Craniofacial Research NIDCR (R21DE023649). Authors acknowledge FAPESP grants and scholarships: Young Investigators Awards Part I # 2012/19078-7 and part II # 2021/06730-7 and # 2019/08693-1, and BEE #2021/08018-2 as well as acknowledge the financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001; Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) projects: CNPq307255/2021-2.

DATA AVAILABILITY STATEMENT

The study was conducted by the Institutional Animal Care and Use Committee approval and national guidelines (Comité d'éthique Anses/ENVA/UPEC #10-0057).

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How to cite this article: Bergamo ETP, de Oliveira PGFP, Campos TMB, et al. Osseointegration of implant surfaces in metabolic syndrome and type-2 diabetes mellitus. *J Biomed Mater Res*. 2024;112(2):e35382. doi:[10.1002/jbm.b.35382](https://doi.org/10.1002/jbm.b.35382)