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May dental implant macro and microgeometry modifications influence peri-implant bone repair in smokers? A randomized clinical trial

Fabiano Ribeiro Cirano[1], Andre Luis Seferian Óbice[1], Felipe Fonseca Girlanda[1], Mabelle Freitas Monteiro[1], Suzana Peres Pimentel[1], Marcio Zaffalon Casati[1] and Mônica Grazieli Corrêa[1*]

Abstract

Background This split-mouth, double-masked, randomized clinical trial aimed at evaluating the impact of different macro geometries and nano topographical modifications on peri-implant bone repair in smokers.

Methods Thirty-two patients who smoked at least ten cigarettes/day, with the need of a single maxillary or mandibular implant bilaterally, received two implants randomly assigned to DA - Dual Acid-Etched implants ($n=32$); HCAN – healing chambers and activated nano surface ($n=32$). Implant stability quotient (ISQ) was evaluated 07, 30, 60, 90, and 120 days after implant placement. Levels of bone and angiogenic markers were quantified in the peri-implant fluid after 07, 15, 30, 90, and 120 days of implant insertion. HCAN implants have a higher ISQ than DA implants at 60 days ($p<0.05$).

Results PLGF levels were lower for HCAN implants than for DA implants at 07-day period ($p<0.05$). Besides, HCAN implants presented higher levels of OPG at 30 days and OPN, BMP-9, FGF-1, PLGF, and VEGF at 90 days, compared to DA implants ($p<0.05$). The levels of EGF were higher for HCAN implants at 15, 90, and 120 days compared with DA implants ($p<0.05$). HCAN implants also showed lower levels of TNF-α at 07 days in comparison to DA implants ($p<0.05$) but had higher levels of DKK1 at 30 days, while DA implants presented higher levels of this marker at 90 days ($p<0.05$).

Conclusion Macro geometry and nano topographical modifications positively modulated the bone and angiogenic factors, resulting in higher production of these markers during early peri-implant bone healing and having a positive effect on implant stabilization in smokers.

Trial Registration RBR-10gjvcyt; date of registration: 06/12/2023 (Retrospectively registered).

Keywords Dental implants, Surface treatment, Bone, Biological markers, Protein array, Biomarkers, Osseointegration, Nanostructures, Biomechanics

*Correspondence:
Mônica Grazieli Corrêa
monica.grazieli@hotmail.com
[1]Departamento de Odontologia, Universidade Paulista (UNIP), Av. Dr. Bacelar, 1212, Vila Clementino, São Paulo 04026-002, Brazil

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Background

Smoking has been considered a risk factor for dental implant failure [1–4] and for peri-implantitis development [5–7]. Moreover, it has been observed higher peri-implant marginal bone loss in smokers than in nonsmokers [8] and heavy smokers may have a higher incidence of implant failure [9–11]. Additionally, epidemiologic studies have reported that smoking reduces bone density and increases bone fracture occurrence, leading to delayed bone repair and altered bone metabolism [12, 13].

Some pathophysiologic mechanisms could help to explain the negative impact of cigarette smoking on peri-implant tissues, including alterations in important bone and angiogenic factors such as nuclear factor kappa-B (RANK), receptor activator of nuclear factor kappa-B ligand (RANKL), and osteoprotegerin (OPG) [RANK–RANKL–OPG system], bone morphogenetic proteins (BMPs), transforming growth factor-β (TGF-β) and alkaline phosphatase (ALP) [14, 15] and reduced the levels of IL-4, -8 TNF-α, and OPG [16]. Additionally, exposure to nicotine leads to a decrease in mRNA levels of OPN, COL-II, Bone morphogenetic protein 2 (BMP-2), bone sialoprotein (BSP), and Core-binding factor alpha-1 (Cbfa-1) [17]. Furthermore, cigarette smoke itself is an exogenous and major source of reactive oxygen species (ROS) (108 organic free radicals per puff in the gas phase and 1019 free radicals per gram in the tar phase) [18–20] that cause cellular oxidative stress which can affect the expression of inflammatory signaling molecules and the differentiation of osteoblasts and osteoclasts [21, 22].

Considering this, the investigation of more predictable implant therapies in smokers could result in new approaches to overcome the harmful influence of smoking on bone healing. Therefore, innovative implant macro geometries with strategic spaces between the implant surface and the surgical bed ("healing chambers") have been suggested as an approach that can benefit the results related to peri-implant repair [23–25]. The biological plausibility of this modification on implant macro geometry is founded on the fact that healing chambers are filled by blood clots immediately after implant installation, contributing to the healing process [25–27]. Healing chamber configurations have been reported as an important aspect of secondary stability and may not affect primary stability [25, 28, 29]. The near contact between the implant surface and the bone bed is substantial for primary stability and subsequent osseointegration [30, 31], but evidence has indicated that this condition may promote impairment on peri-implant bone healing by the extensive bone resorption that occurs around the implant during healing [29, 32, 33]. It is important to highlight that bone formation in the presence of healing chambers occurs through intramembranous ossification with new bone formation directly on the implant surface and at the surgically instrumented bone wall, which reduces appositional ossification and bone resorption due to the reduction of compression necrosis [34, 35]. In this type of bone formation, the healing chambers are rapidly filled by woven bone, which is subsequently replaced by lamellar bone surrounding multiple primary osteogenic structures throughout the healing chamber volume [34, 35].

Additionally, chemical modifications on the implant surface, such as the presence of hydroxyapatite (HA), aim at accelerating the bone response and the possibility of functional rehabilitation in a shorter time by promoting a faster osseointegration [36, 37] due to the osteoconductive impact of the surface [26]. In the past, the HA layer was thick and could be detached from the implants over time, leading to several clinical complications. However, the insertion technique in a manometric scale has been developed, promoting effective cell integration without signs of foreign body reaction. Thus, the nano topographical modifications of implants and their chemical composition have a synergistic role in accelerating osseointegration [38]. Nano-scale changes have promoted pre-osteoblast differentiation, with higher levels of osteocalcin and osteoprotegerin expression in vitro [39]. Besides, an in vivo study showed that nanostructure implants presented higher bone-to-implant contact than polished implants [40].

In this regard, implants with optimized macro geometry associated with nano topographical modifications could benefit the therapy with dental implants in the presence of smoking.

To our knowledge, this study evaluated for the first time the influence of an implant with modified macro design and nano topographical modifications based on the presence of a healing chamber and activated nano surface in the pattern of peri-implant repair under smoking conditions. A better understanding of biomechanical aspects and molecular mechanisms related to using modified implant macro designs on smokers could support the use of this strategy to favor the rehabilitation of dental implants in this condition.

Methods

Population screening

The prospective, randomized, double-masked, split-mouth, and controlled clinical study population was recruited from patients seeking dental treatment at Paulista University, São Paulo, Brazil. The inclusion criteria in this study consisted of smokers (>10 cigarettes/day) aged between 18 and 65 years; ASA classification II; healthy periodontal status; the presence of at least 20 teeth in the oral cavity and, bilateral and homologous unitary prosthetic space. The exclusion criteria were: the presence of systemic diseases that could interfere with

bone regeneration (diabetes, arthritis, hypothyroidism, hyperparathyroidism, and osteoporosis); pregnancy or breastfeeding; use of medications that counter-indicated the performance of surgical procedures or that could alter bone regeneration around implants (e.g., anti-inflammatory and bisphosphonate drugs).; absence of keratinized tissue at the implant insertion sites (<2 mm; may interfere with hygiene around the implants) [41]; need for bone or tissue grafts.

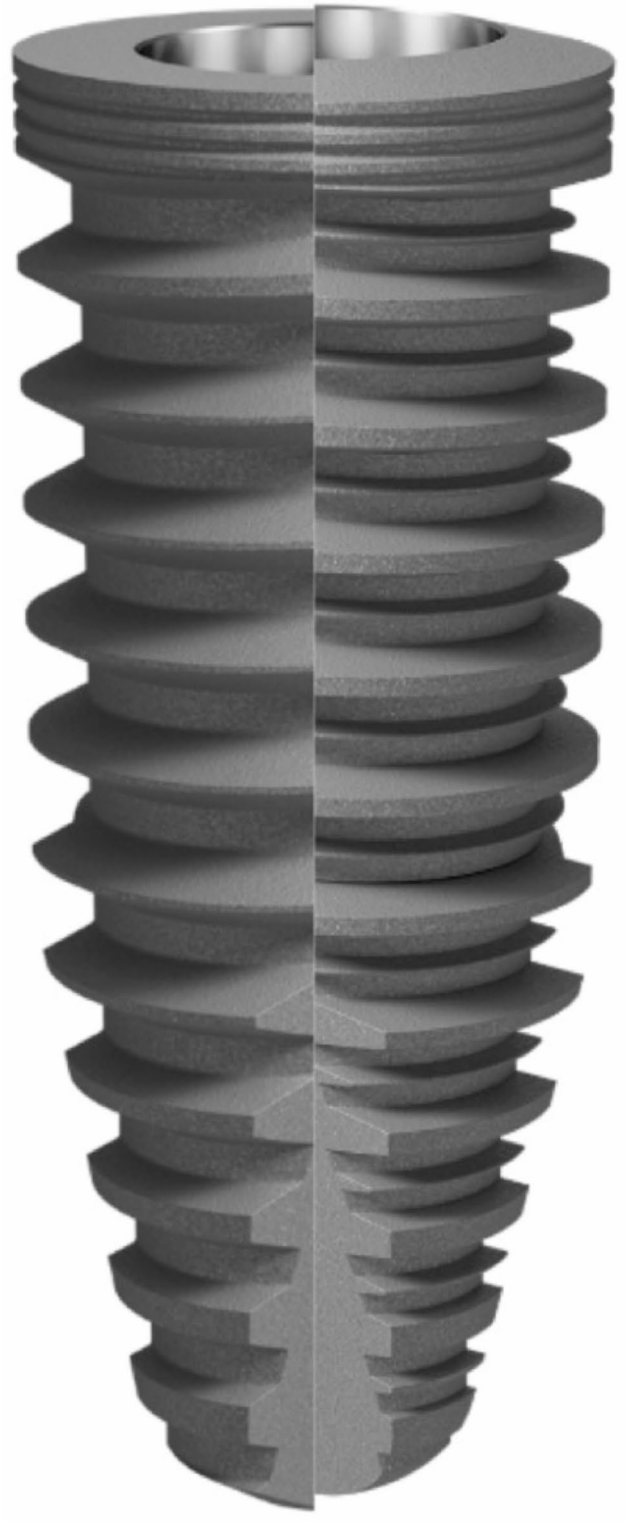

Fig. 1 Schematic illustration of DA and HCAN implants

Sample size calculation

The number of patients included in the present study was based on previous studies that had found significant differences in the levels of bone, angiogenic, and inflammatory markers in peri-implant fluid [42, 43]. The calculation yielded a minimum sample size of 18 participants per group. Considering that several individuals and implants may be lost throughout follow-up, 32 individuals were included in each group.

Randomization

Patients were randomly assigned, using a computer-generated list generated a priori (SAS 9.3 software; SAS Institute Inc., Cary, NC, USA). by a team member not involved in the clinical procedures, to one of the two treatment modalities: DA - Dual Acid-Etched implants – single thread design (SW - S.I.N. IMPLANT SYSTEM) (n=32); HCAN – healing chambers + dual thread design [thread within thread profile] and activated nano surface - nano-sized HA according to the Promimic HAnano™ method [38] (UNITITE - S.I.N. IMPLANT SYSTEM) (n=32).

Treatment protocol – implant placement

All the surgeries and post-operative follow-ups were performed by the same surgeon (A. L. S. O.) at the Dentistry Clinic of Paulista University, São Paulo, Brazil. All the patients received two cone morse connection implants in one stage (S.I.N. IMPLANT SYSTEM, São Paulo, Brazil), which had their prosthetic platform positioned 2 mm below the bone crest after the installation of the implants with insertion torque varying from 30 to 45 N. One side received a DA (Dual Acid-Etched implant), and the other, an HCAN (healing chambers and activated nano surface). Hexagonal conical abutments and conical abutment protectors (Implacil de Bortoli, São Paulo, Brazil) were installed (Fig. 1).

After anesthesia, a mid-crestal incision on the bone crest was performed, and a mucoperiosteal flap was shifted. The site was prepared following the manufacturer's instructions, and the intermediate implants and abutment protectors were installed. Uninterrupted sutures were performed using 4.0 nylon (Nylon, Ethicon, Somerville, New Jersey, USA). Amoxicillin (2 g administered one hour before the procedure), sodium dipyrone (500 mg every six hours for two post-operative days), and mouthwash with 0.12% chlorhexidine digluconate (every

12 h for seven days) were indicated. All patients received single prostheses within 90 days of implant placement.

Dental implant stability assessment

The implant stability quotient (ISQ) was determined by resonance frequency measurements using Osstell® (Integration Diagnostics AB, Göteborg, DAeden) at implant placement and 30, 60, 90, and 120 days later. The measurements were performed in triplicate by the same examiner (A.S.O).

Fluid collection and evaluation of osteoblastogenic and angiogenic markers

Peri-implant fluids collection was performed by a blinded examiner in each group, using filter paper strips (Periopaper; Oraflow, Plainview, New York, USA) at baseline, 15, 30, 90, and 120 days after implant installation. The levels of bone and angiogenic mediators (of Dickkopf-1 (DKK1), osteoprotegerin (OPG), osteopontin (OPN), vascular endothelial growth factor (VEGF), Sclerostin (SOST), epidermal growth factor (EGF), fibroblast growth factor (FGF), placental growth factor (PlGF), osteocalcin (OC), bone morphogenetic protein-9 (BMP-9), tumor necrosis factor (TNF) alpha and receptor activator of nuclear factor-κB ligand (RANKL) were determined using a specific kit (HAGP1MAG-12 K, HRNKL-51 K, HRNMAG-51 K, Millipore Corporation, Billerica, MA, USA), and measured with a multiplex instrument (MAGpixTM; MiraiBio, Alameda, CA, USA), following the manufacturer's instructions. The samples were individually evaluated and adjusted for fluid volume, and the concentrations were estimated from the standard curve using a five-parameter polynomial equation and specific software (Xponent® Millipore Corporation, Billerica, MA, USA). The mean concentration of each biomarker was calculated and expressed as pg/ml.

Statistical analysis

The primary outcome variable was the biomarker levels. The secondary outcome was ISQ. Initially, the data were submitted for normality tests (Shapiro-Wilk test). Once the data distribution was abnormal, ISQ data and immunoenzymatic markers were submitted to the Wilcoxon and Friedman test (inter and intra-group analysis, respectively). An experimental significance level was established at 5% for all statistical analyses. The biostatistician was blinded to the treatment allocation of the quadrants. The analyses were performed using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

Results

Thirty-two individuals were included in the present study, and no patients withdrew during the experimental stage (Fig. 2). Table 1 shows the data concerning sex, age, and implant distribution, and no statistically significant differences were observed ($p>0.05$).

Resonance frequency analysis

In the resonance frequency analysis, the implant stability quotient (ISQ) presented higher values for HCAN implants at a 60-day time-point ($p<0.05$). Intragroup analysis showed higher ISQ values at a 120-day time-point compared to 30-day periods in the HCAN group ($p<0.05$). The DA group presented higher values at 90 and 120 days than at 30-day periods ($P<0.05$). The data regarding ISQ are summarized in Fig. 3.

Immunoenzymatic markers levels

The immunoenzymatic results are illustrated in Fig. 4. An immunoenzymatic assay for bone and angiogenic markers showed that, for intergroup analysis, HCAN implants demonstrated higher levels of DKK1 at a 30-day time point ($p<0.05$). In comparison, DA implants presented higher levels of this marker at a 90-day time-point ($p<0.05$). HCAN implants presented higher levels of OPG at 30 days and OPN, BMP-9, FGF-1, and VEGF at 90 days, compared to DA implants ($p<0.05$). The levels of EGF were higher for HCAN implants at 15-, 90- and 120-day periods compared to DA implants ($p<0.05$). HCAN implants also showed lower levels of PLGF and VEGF at the 07-day time-point and lower levels of SOST at the 30-day time-point in comparison to DA implants ($p<0.05$). However, the levels of PLGF were higher for HCAN implants at a 90-day time-point ($p<0.05$). HCAN implants had lower levels of TNF-α at 07 days' time-point ($p<0.05$). No statistical difference intergroup exists for OC and RANKL ($p>0.05$).

Regarding intragroup analysis of HCAN implants, higher levels of DKK1 were observed at 30- and 120-day time-points compared to 07 days and at 30 days compared to 15-day period ($p<0.05$). OPG, OPN, BMP-9, and VEGF at 30-, 90- and 120-days' time-point compared to 07 days ($p<0.05$). Besides, the levels of VEGF were higher at the 15-day time-point compared to the 07-day time-point ($p<0.05$), and the period showed elevated levels of OPG when compared to the 15-day time-point ($p<0.05$). HCAN implants presented higher levels of EGF and PLGF at 15, 30, and 90 days-period compared to the 07-day time-point ($p<0.05$). The levels of FGF-1 were higher at 30 and 90 days than at 15 days ($p<0.05$). The levels of TNF-α were higher at the 30 and 90 periods when compared to the 07-day time-point and at 30 days compared to the 15-day time-point ($p<0.05$). RANKL levels were higher at 90 days when compared to 15 days ($p<0.05$). HCAN implants did not present intragroup differences for OC and SOST ($p>0.05$).

Considering the intragroup analyses for DA implants, higher levels of DKK1 were observed at 90 days-period

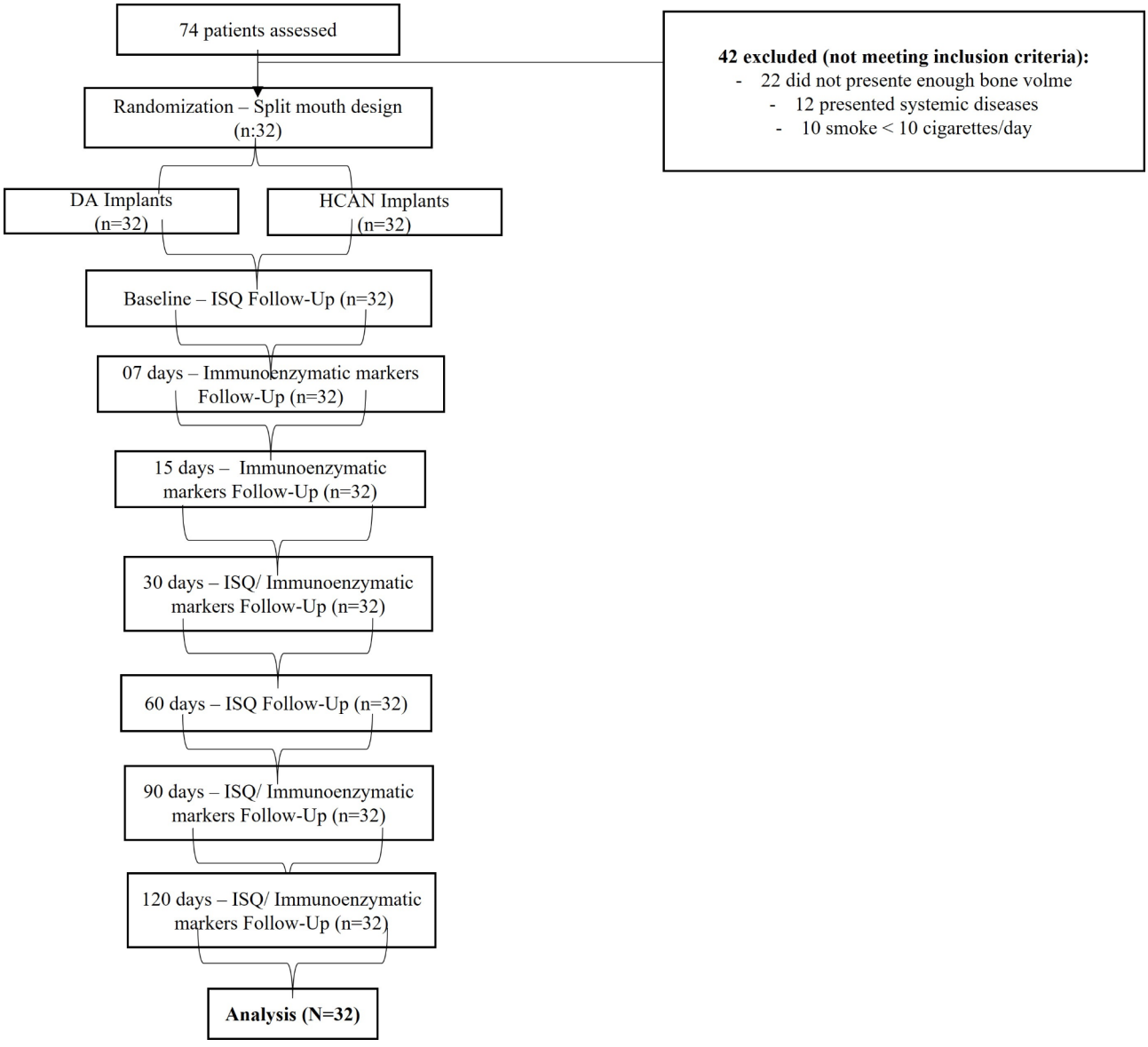

Fig. 2 Flowchart of the study showing the patients enrolled in the pre-study phase and the selection of individuals for the study phase

Table 1 Data concerning by sex, age, and implant distribution

Age (Years) (mean – SD)	**43,94 (9.07)**
Gender (Female) (%)	53,13
Ethnicity (White) (%)	65,63
Cigarretes/day (mean – SD)	16,03 (5.49)
Smoker time (years) (mean – SD)	11 (4.06)
Implants distribution in Maxila (n)	34
Implants distribution in Mandibula (n)	30

Gender and race parameters were evaluated using Fisher's exact test (α = 0.05). The other parameters were evaluated by Student's t-test for 2 different samples (α = 0.05). There were no differences between groups in any parameter

when compared to 07 and to 15 days time-point ($p<0.05$). DA implants also showed higher levels of OPG at 90 and 120 days compared to 15 days ($p<0.05$). Higher levels of FGF-1 were observed at 30- and 90-day time points compared to 120-day periods ($p<0.05$). Higher levels of SOST and PLGF were observed at 30 days' time-period compared to the 07 and 120 days-period ($p<0.05$). DA implants had higher levels of BMP-9 at 30 days-period compared to 07 days ($p<0.05$). The levels of EGF were higher at 30 and 90 days when compared to 120 days-period ($p<0.05$). It also showed elevated levels of FGF-1 and RANKL at 30, 90, and 120 days compared to the 07-day time-point ($p<0.05$). The levels of RANKL were also higher at 30- and 120-day time-points compared to the 15-day ($p<0.05$). DA implants did not present intragroup differences for TNF-α, OC, OPN, and VEGF ($p>0.05$).

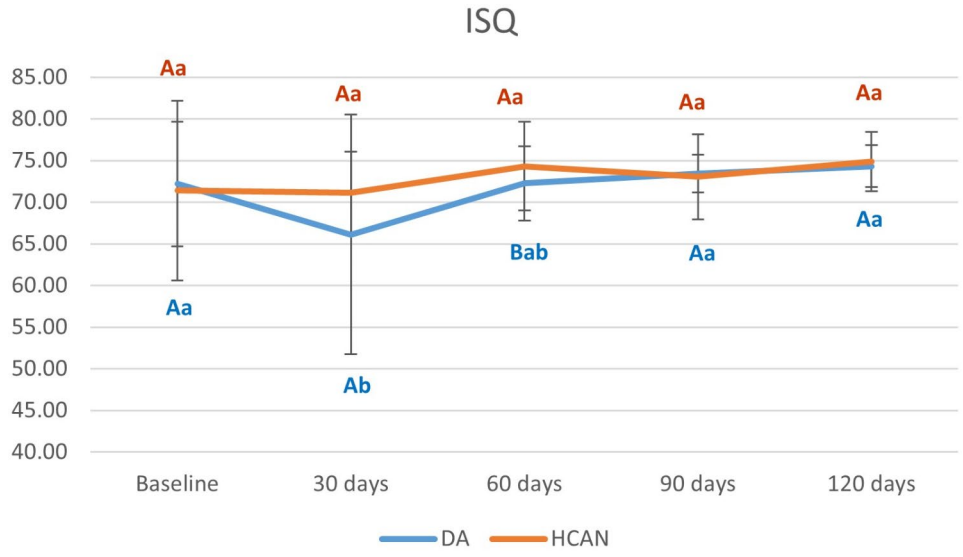

Fig. 3 Graphic illustrating means and standard deviations of Implant stability quotient at baseline, 30, 60, 90, and 120 days. Different letters indicate statistical significance. The uppercase letter compares the difference between groups (within the same time; Wilcoxon Test; $p < 0.05$); Lowercase letters compare longitudinal data within the same group; Friedman test; $p < 0.05$). Red letters refer to the HCAN group, while blue letters refer to the DA group

Discussion

Modifications of macro geometry and nano topography of implants, such as healing chambers and nano-activated changes, have been suggested as an approach to enhance peri-implant repair, to accelerate osseointegration, leading to higher bone-implant contact and implant stability, suggesting the possibility of faster prosthetic loading. In the face of that, this could be an interesting approach for patients with compromised bone quality and repair, such as smokers. Within the author's knowledge, there is no clinical study evaluating peri-implant bone healing and stability using an implant with a modified macro design and activated nano surface under smoking conditions. The present study showed higher levels of bone and angiogenic factors and higher implant stability at early bone healing phases using geometric modifications.

In this trial, resonance frequency analysis demonstrated intergroup difference at 60 day period, with higher levels of ISQ for HCAN implants; it can be suggested that the implant modifications on the macro geometry and nano topography could enable earlier implant stability in the presence of a factor such as smoking that leads to impaired bone repair. A recent trial comparing implant stability in smokers and nonsmokers showed a decrease in secondary stability with smoking habit, with a difference of 3.61 points in the mean variation of implant stability for smokers and nonsmokers [44]. In line, another study comparing implant stability between nonsmokers and heavy smokers demonstrated that bone healing around dental implants was decreased due to a reduced healing speed, indicating that the time to apply implant loading in heavy smokers is important [45]. In our study, although the ISQ differed between groups only at 60 day period, the levels of implant stability in both groups were stable until 120 days, suggesting that the modifications on the macro geometry and nano topography could have helped contribute to increased bone healing, preventing ISQ levels decrease throughout the time and that the implant loading could be applied safely for HCAN group. Dual acid-etched implants have shown a higher success rate in lower-quality bone, reducing healing time and allowing earlier implant loading and rehabilitation [46]. Another study showed a higher success rate for dual acid-etched implants than machined implants in poor-quality bone (12% difference) [47]. Although these studies did not compare the success rate of dual acid-etched implants between smoker and nonsmoker patients, it is known that smoking condition affects bone density [12, 48] and bone quality [49], and these results could be extrapolated to a clinical situation where smoking is present. These findings could help explain why ISQ levels differed only at the 60-day time point.

Important bone-related factors such as DKK1 and OPG had elevated levels in the presence of macro and nano topographical modifications 30 days after implant installation. In contrast, DKK1 levels were higher in DA implants at 90 days. DKK1 is well-known for suppressing osteoblast differentiation and facilitating osteoclastogenesis [50]. However, DKK1 is also related to correct skeletal development and homeostasis [51–53]. Conversely, OPG inhibits the differentiation and function of osteoclasts [54], modulating bone maturation and resorption [55]. On the other hand, there is no difference between the groups regarding RANKL levels, but lower levels of TNF-α were found in HCAN implants at 07 days. Interestingly, a previous study of our research group showed

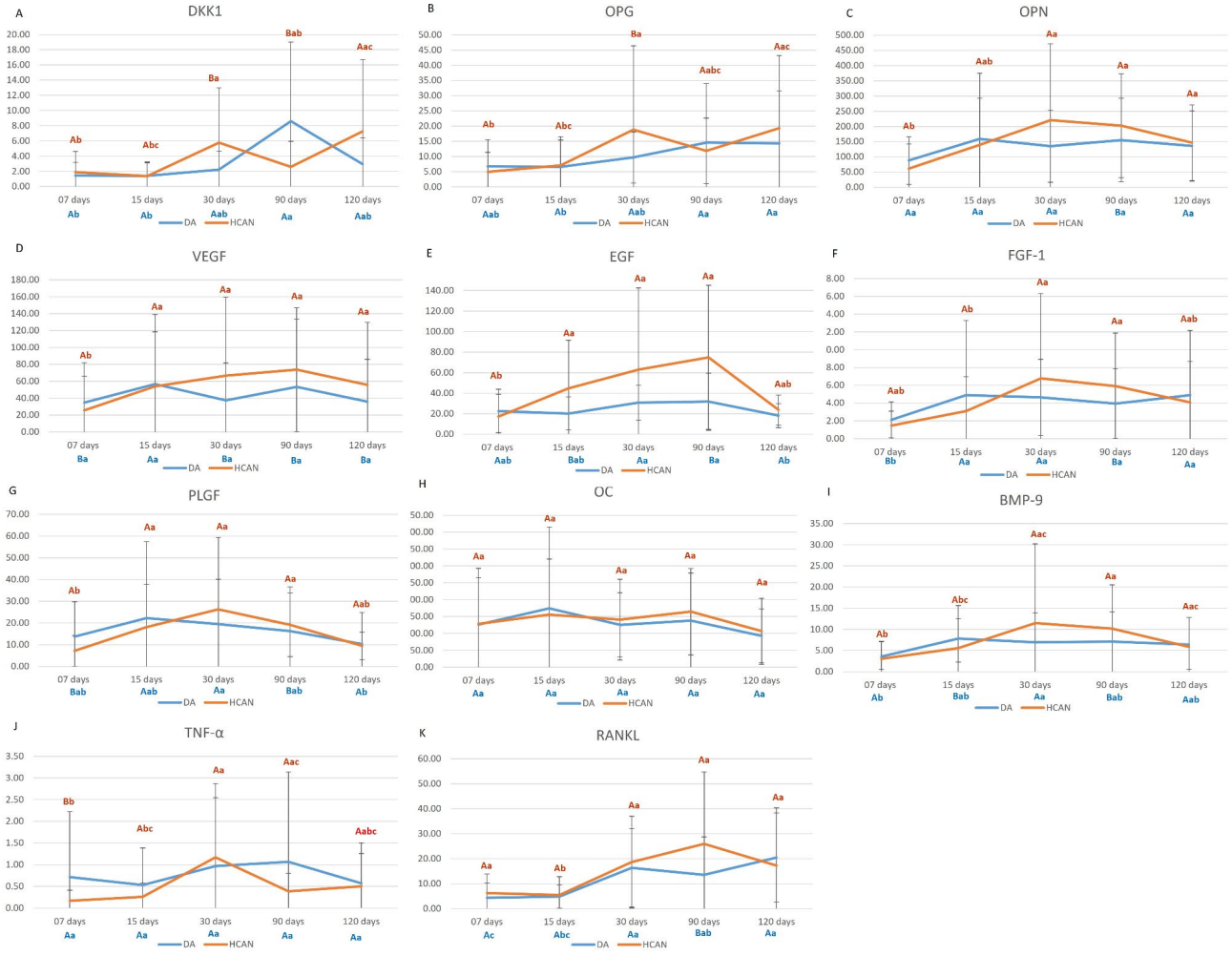

Fig. 4 Levels (means and standard deviations) of bone and angiogenic factors in the peri-implant fluid in the HCAN and DA groups. **A**- Dickkopf-1 (DKK1); **B**- Osteoprotegrin (OPG); **C**- Osteopontin (OPN); **D**- Vascular endothelial growth factor (VEGF); **E**- Epidermal growth factor (EGF); **F**- fibroblast growth factor (FGF); **G**- Placental growth factor (PlGF); **H**- Osteocalcin (OC); **I**- Bone morphogenetic protein-9 (BMP-9); **J**- Tumor necrosis factor (TNF) alpha; **L**- Receptor activator of nuclear factor-κB ligand (RANKL). Different letters indicate statistical significance. The uppercase letter compares the difference between groups (within the same time; Wilcoxon Test; $p < 0.05$); Lowercase letters compare longitudinal data within the same group; Friedman test; $p < 0.05$). Red letters refer to the HCAN group, while blue letters refer to the DA group

higher levels of mRNA of RANKL/OPG in the bone tissue around implants inserted in rats submitted to cigarette smoking inhalation than in non-smoking animals [56]. An in vitro study showed that nano-scale changes enhanced pre-osteoblast differentiation, which showed significantly higher levels of OC and OPG expression [39]. However, the present study did not observe intergroup differences in OC levels. Even though it is known some pathways through smoking negatively affect some bone factors, such as the RANKL/OPG system [57], no data is available about the modulation of DKK1 by cigarette smoking. As regards TNF-α levels in smokers, it was verified higher levels in the gingival crevicular fluid when compared to nonsmokers [58], and it could be attributed to the potent inhibitors contained in the smoke of both gene expression and protein production for this marker [59].

In line, the concentration of OPN and EGF was higher at 90-day periods in the presence of macro and microgeometry modifications. OPN is related to binding basic elements to the extracellular bone matrix and bone mineralization [60] and regulates angiogenesis as a response to cell stress, cell adhesion, chemotaxis, and cell motility [61]. Interestingly, osteoblasts seeded onto HA-treated surfaces had higher expression of OPN when compared with the double acid-etched surface [62]. Convergently, EGF acts in differentiation and mineralization of osteoblast differentiation, DNA synthesis, growth and proliferation of mesenchymal cells, angiogenesis, stimulation of vascular permeability, tissue proliferation, and faster tissue regeneration during the initial peri-implant bone

regeneration phases [63–65]. This marker was also elevated for HCAN implants at 15 and 120 days after implant placement. These results are important findings of this trial once bone density in the presence of smoking is reduced [49], and this may favor both bone quality and anticipated implant loading time.

BMP-9 and FGF-1 presented higher concentrations using macro and nano topographical modification at a 90-day time-point. BMP-9 has the highest osteogenic activity among bone morphogenetic proteins, which differentiate mesenchymal cells into osteoblasts [66, 67]. The higher levels of the marker FGF-1 probably contributed to faster bone healing around HCAN implants since its function includes fibroblasts, endothelial cells, and osteoblasts proliferation [68, 69], induction of angiogenesis, and the expression of proteases, growth factors, and integrins involved in angiogenesis [70]. The elevated concentration of both markers at early phases of healing may accelerate bone healing due to their action on regeneration, chemical attraction, proliferation, and cell differentiation into osteoblasts [71].

Interestingly, the levels of VEGF and PLGF were elevated in the presence of implant design modifications at 90 90-day periods. Both are angiogenic factors linked to osteoclasts, osteoblasts, and chondroblasts differentiation [72], and proliferation, migration, and survival of endothelial cells [73]. These results indicate pronounced angiogenic activity with modifications of the macro and micro geometry of implants. However, this result did not favor the healing process around the implants, once ISQ levels did not differ between groups at this point.

In line, the results concerning bone and angiogenic markers show, in a general way, higher concentration of most of the studied markers at 30- and 90-day periods, which can help to explain the results regarding ISQ level (higher values for HCAN implants at 60 days). In this study, bone and angiogenic markers were not measured at the 60-day time point, and maybe if the measurement had been done, a higher concentration of the markers could also have been observed at this point. Another interesting point to be highlighted is the reduction in most of the markers studied from 90- to 120 days, which could be associated with implant loading at the 90-day time point. In line, Prati et al. [74] evaluated the pattern of bone markers released during peri-implant bone repair of immediately loaded and unloaded implants, observing earlier peaks of TGF, OPG, OPN, and parathyroid hormone in loaded implants.

The benefits of implant design with modified macro geometry have been reported in some investigations [28, 75]. Recently, a preclinical trial demonstrated histological and clinical evidence supporting the safety and efficacy of an implant design with modified macro geometry capable of obtaining promising primary and secondary stability in alveoli after immediate extraction, without the use of bone grafts [75]. Studies were also carried out to point out the interference of the use of chemical agents on the implant surface and showed promising results concerning the macro geometry of the implants, regardless of the surface treatment given to the implants [28]. Recent animal studies have proven the effectiveness of a modified macro geometry after a short initial repair period using a counter-torque method [28].

It has been shown the positive effect of nano topography in cell activity due to the interactions among cell, matrix, and substrate associated with cell signaling happen at the nanometer stage [76], which results in the regulation of cell migration, proliferation, adhesion, spreading, and differentiation and both gene and protein expression [77, 78]. This results in a biological response to the material and accelerated healing and osseointegration [79, 80].

The results of this trial are important to elucidate the molecular mechanisms in early phases of bone healing around implants with macro geometry and nontopographical modifications, showing the possibility of a faster healing process and implant loading and allowing a faster rehabilitation process in smokers patients. However, further studies are necessary to verify the behavior of tissues after implant loading in terms of clinical behavior, verifying clinical parameters, the release of inflammatory markers through immuno-enzymatic assays, and bacterial colonization throughout the time. Additionally, diversifying assessment methodologies could provide a more comprehensive understanding of bone repair processes.

Taken together, the results of the present study showed that macro design modification and nonactivated surface could enhance bone repair during the early phases of osseointegration, contributing to lower bone resorption due to the positive modulation of bone and angiogenic factors resulting in higher production of these markers during early peri-implant and higher implant stability in early phases of repair in the presence of the adverse effects of cigarette smoking. Thus, in conclusion, the implant macro geometry modification associated with nonactivated surface positively modulated bone and angiogenic factors, resulting in higher production of these markers during early peri-implant bone healing and positively affecting implant stabilization in smokers.

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Author contributions
FC made substantial contributions to the conception and to design the work; ALSO made substantial contributions to the acquisition, analysis, and interpretation of data; FFG made substantial contributions to the acquisition, analysis, and interpretation of data; SPP made substantial contributions to the conception and to design the work; and to the interpretation of data;

MFM made substantial contributions to the conception and to design the work; and to the interpretation of data; MZC made substantial contributions to the conception and to design the work; and to the interpretation of data; substantively revised the work; MGC made substantial contributions to the conception and to design the work; and to the interpretation of data; substantively drafted the work; All authors have approved the submitted version (and any substantially modified version that involves the author's contribution to the study); and agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All the participants were informed about the nature, potential risks, and benefits of their participation in the study, and signed an informed consent form. This study was approved by the Research Ethics Committee of Paulista University (80794517.8.0000.5512 – Rebec -BR-10gjvcyt - Retrospectively registered) and presented a CONSORT-based design.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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