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ORIGINAL ARTICLE

Impact of resveratrol in the reduction of the harmful effect of diabetes on peri-implant bone repair: bone-related gene expression, counter-torque and micro-CT analysis in rats

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ABSTRACT

Objective: Investigate the impact of resveratrol (RESV) on peri-implant repair and its effect on bone-related markers in rats with induced diabetes mellitus (DM).

Material and methods: Ninety rats were divided into: DM + RESV ($n = 18$); DM + placebo (PLAC) ($n = 18$); DM + insulin (INS) ($n = 18$); DM + RESV + INS ($n = 18$); Non-DM ($n = 18$). Diabetes was induced by streptozotocin. One screw-shaped titanium implant was inserted in each tibiae of animals. Treatments were administered during 30 days. After, one of the implants was removed for counter-torque and the peri-implant tissue was collected for mRNA quantification of BMP-2, OPN, Runx2, Lrp-5, Osx, β-catenin, Dkk1, OPG, and RANKL by Real-time PCR. The other tibia was submitted to MicroCT analysis to measure: bone volume (BV/TV), trabecular thickness (Tb.Th) and bone-implant contact (BIC).

Results: Higher counter-torque values were observed for implant removal in DM + RESV, DM + RESV + INS and Non-DM groups when compared to DM + PLAC ($p < .05$). Augmented Tb.Th was observed in DM + RESV and Non-DM when compared to DM + PLAC group ($p < .05$), whereas higher BIC was detected in DM + RESV, DM + RESV + INS and Non-DM animals when compared to DM + PLAC ($p < .05$). Levels of RANKL were downregulated by the RESV and/or INS therapy, whereas only the association of RESV and INS upregulated the levels of Runx2 ($p < .05$).

Conclusions: The therapy with RESV may favour peri-implant bone repair improving bone formation around implants.

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Diabetes mellitus; dental implants; gene expression; osseointegration; resveratrol

Introduction

Diabetes mellitus (DM) is an important global disease with 8.2% of adults (387 million people) living with this condition and the International Diabetes Federation projected that this number will rise beyond 592 million in 2035 [1]. While prevalence of diabetes is augmenting, deaths from diabetes have decreased, probable due to advances in clinical treatments of the complications of DM [1].

Thus, considering the increasing in the life expectancy in this patient profile, likely rehabilitations with dental implants will become more necessary, especially taking into account the raised incidence of periodontitis and consecutive tooth loss in diabetic individuals [2]. The use of rehabilitations with dental implants may be considered an important therapeutic alternative to ensure adequate masticatory function and satisfactory nutritional ingestion, crucial aspects in the treatment of diabetics, besides to improve the life quality of these patients [3].

Nevertheless, evidences have pointed out that hyperglycaemia promotes harmful impact on bone healing around implants and may negatively influence the dental implant stability [3,4], besides to damage the bone quality throughout healing processes [5], which may contribute to the elevated risk of peri-implantitis and to the higher level of implant failure in this patient profile [6]. In this context, evidences have supported that hyperglycaemia may negatively influence the host osteoimmunoinflammatory response, modifying the molecular pathways related to the bone repair in diabetics [7,8].

Innumerous investigations have focussed on therapies to overcome the harmful effects of hyperglycaemia in the healing of bone surrounding implants, including implant surface modification, parathyroid hormone therapy, use of hypoglycemiant agents, mesenchymal stem cell management, hyperbaric oxygen treatment and doxycycline therapy [9–13]. Nevertheless, most of these therapies have presented not predictable outcomes on osseointegration of implants and have not been recognized as therapeutic alternatives to revert or contain the negative impact of DM on peri-implant bone healing, besides to be, in some cases, related to side effects for using on diabetic conditions.

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In order to obtain a more predictable therapy with minimal adverse effects, interest concerning the effect of naturally occurring agents on the repair around implants in diabetic circumstances have increased and some substances as curcumin and berberine have demonstrated promising influence on peri-implant bone repair under hyperglycaemic situations [14,15]. Resveratrol (trans-3,4,5′-trihydroxystilbene) (RESV) is one of these active substances derived from plants and food with innumerous biological properties [16–19], including inhibitory impact on osteoclast differentiation and potential for improving bone formation [8,20–22]. In this context, studies have also demonstrated the influence of RESV in positively modulate relevant osteoblastogenic markers, as Runx2, OPN and BMP-2 [8,20,23,24]. Other important bone-related molecules, such as RANKL and OPG, may also be impacted by RESV-therapy in different biological conditions related to bone repair [23,25,26]. Importantly, RESV also exerts relevant therapeutic action in glycemic control [27], supporting that this natural agent may be used as adjunctive supplementation in the management of DM, with promising effects to prevent bone density loss in patients with DM [28]. However, the influence of this phytochemical on the peri-implant bone repair under diabetic conditions is not yet known. Then, this investigation was designed to determine the influence of systemic administration of RESV in reversing the harmful effect of hyperglycaemia on bone healing around implants.

Materials and methods

Animals

This experimental study was approved by the Animal Care and Use Committee of University (Permit Number: 226/14). The animal cohort was composed of 90 10-week-old male Wistar rats, weighing 334 ± 69 g at the beginning of the study. The animals were acclimatized for 15 days before experiments in the Bioterium of University. Animals were maintained in temperature-controlled cages, exposed to a 24-h light–dark cycle of equal time, and had food ad libitum (Labina; Purina 1, Paulinia, SP, Brazil) and free contact to water.

Treatment groups

Animals were assigned to five experimental groups: non-diabetic animals treated with placebo (Non-DM; $n = 18$); animals with induced DM and treated with placebo (DM + PLAC; $n = 18$); animals with induced DM and treated with resveratrol (DM + RESV, $n = 18$); animals with induced DM and treated with insulin (DM + INS; $n = 18$; and animals with induced DM treated with both resveratrol and insulin (DM + RESV + INS; $n = 18$). The administration of resveratrol (10 mg/Kg) and placebo was performed *via* gavage during 30 days after surgery procedures [22]. A stock solution of resveratrol (R5010-500MG, Sigma-Aldrich Ltda., São Paulo, SP, Brazil) was prepared in Tween-80 (P4780-100 ML, Sigma-Aldrich, São Paulo, Brazil) and further diluted in water to working concentrations. The placebo solution was composed of the same quantities of tween and water as utilized in the preparation of resveratrol. Insulin was administered by subcutaneous injection of neutral protamine harguerdon (NPH insulin; Biohulin NU-1,00,100 IU/ml) diluted in 0.9% NaCl (5.5 ml to 3.5 ml at 6:00 p.m. and at 6 a.m., daily for 30 days following surgery [29].

Experimental induction of DM

The rats were fasted for 14 h prior to DM induction. Diabetes was induced in 72 animals by intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich, St Louis, MO, USA) (60 mg/kg) dissolved in citrate buffer (0.01 M, pH 4.5) [8]. Non-DM animals received intraperitoneal injection of equal volume of 0.1 mol/L citrate buffer. Following 72 h, blood samples were collected from the tail of the animals and inserted in test strips for glucose analysis using a glucose metre (Accu-Check Active®, Roche). Rats that presented levels of glucose above 300 mg/dL were considered diabetics.

Implant placement

Two titanium implants were inserted in each animal, one in each tibia. After anaesthesia, an incision of approximately 10 mm was performed, and the tibiae were exposed by blunt dissection. Bicortical implant beds were drilled using a rotary speed of 1500 rpm. A screw-shaped, commercially available pure titanium implant, 4.0 mm in length and 2.2 mm in diameter (Implacil de Bortoli, São Paulo, SP, Brazil), was inserted in each tibiae until the screw threads had been totally fixed in the bone cortex [20] (Figure 1). The soft tissues were replaced and sutured.

Post-Operative period

The animals were examined regularly during the experiment to evaluation of possible clinical or toxicological symptoms. After thirty days following the start of the experiment, the animals were euthanized by CO_2 inhalation. Then, the tibiae were dissected to access the implants: one of the implants was removed for a torque force assessment and, subsequently, the peri-implant tissue was stored in RNAlater (Ambion Inc., Austin, TX, USA) for gene expression analysis. The other tibiae (including the inserted implant) was removed and stocked in 70% alcohol for analyses by microCT scans.

Counter torque analyze

Torque force assessment for the removal of implants was achieved using a torque metre with a scale range of 0.1–10 N/cm and divisions of 0.05 N/cm (Mark-10, BGI, CA, USA). A wrench was inserted to the implant head to apply torque in the reverse direction of implant placement until rupture of the bone-implant interface was signalled by rotation of the implant. The torque force value achieved was

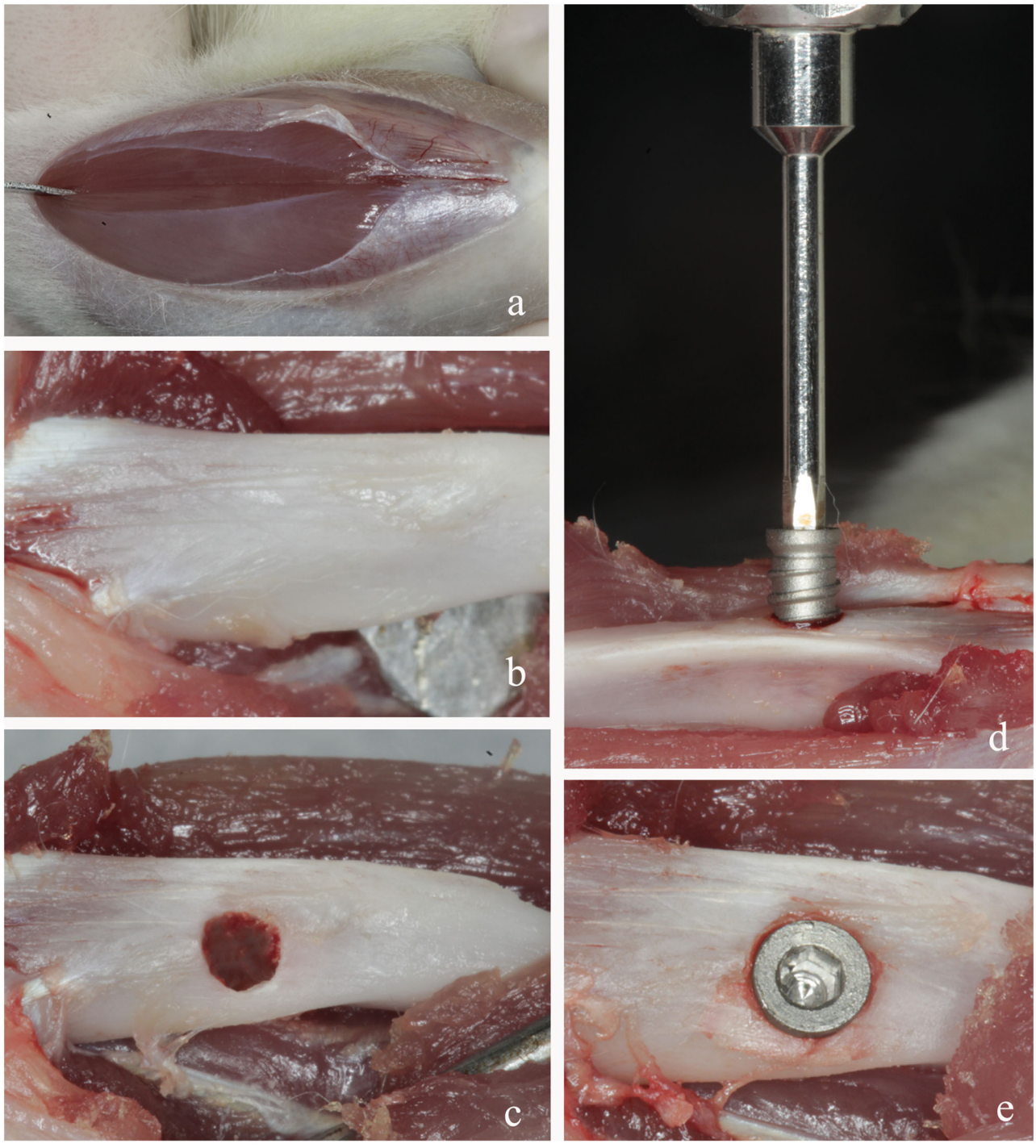

Figure 1. (a) Incision. (b) Surgical exposition of bone surface of the tibiae by blunt dissection. (c) Bicortical implant beds. (d) Insertion of titanium implant into tibiae. (e) Implant completely placed until all screw threads had embedded into the bone cortex.

considered as the torque required for the interruption of osseointegration [30].

Microcomputed tomography analysis of implants

Non-demineralized specimens were scanned by a cone-beam microcomputed tomography (micro-CT) system (Skyscan 1172, Bruker, Kontich, Belgium). The x-ray generator was operated at an accelerated potential of 50 kV with a beam current of 200 mA and an exposure time of 650 milliseconds/projection. Images were concluded with a voxel size of $6 \times 6 \times 6$ mm. The generated three-dimensional models were rotated into a standard position until the implant had its long axis vertically positioned, using an appropriate software (Data Viewer v.1.5.0, Bruker, Kontich, Belgium). In the transaxial axis, the volume of interest (VOI) was then defined, including the bone tissue internal and external to the implant threads. Volumetric analyzes were done with CT-Analyzer® software (CT-Analyzer®, version

1.13.5.1+, Bruker, Kontich, Belgium). In each image, 214 standardized slices equivalent to the extension between the first and last threads present in the implant body were selected. The following parameters were assessed: (a) Bone volume (BV): percentage of VOI filled with bone tissue; (b) Trabecular Thickness (Tb.Th): thickness (mm) of the bone trabeculae present in the VOI; and (c) Bone-implant contact (BIC): percentage of bone tissue in contact with implant surface [31,32]. All evaluations were performed by a blind and calibrated examiner (P.H.F.S.). For calibration, one-third of the sample was assessed in two time periods with a 48-hour interval. The intraclass correlation coefficient (ICC) was used to determine the reproducibility of the examiner in the two evaluations performed. ICC values greater than 90% were considered to guarantee the calibration of the examiner.

Analysis of gene expression

Peri-implant samples designed to gene expression assays and stored in RNAlater solution at −70 °C were evaluated for mRNA levels quantification of the molecules by real-time polymerase chain reaction (qRT-PCR): bone morphogenetic protein-2 (BMP-2), ostepontin (OPN), osteoprotegerin (OPG), receptor activator of NF-ĸB ligand (RANKL), runt-related transcription factor-2 (Runx2), osterix (Osx), β-catenin (β-catenin), low-density protein receptor-related protein 5 (Lrp-5), and Dickkopf-related protein-1 (Dkkl-1). Primers were designed using probe-design software (Light-Cycler Roche probe design software, Diagnostics GmbH, Mannheim, Germany). The amplification profiles, primer sequences, and lengths of PCR products are exposed in Table 1. The qPCR reactions were performed in a real-time PCR apparatus (LightCycler® 96 Instrument, Roche Diagnostics GmbH, Mannheim, Germany) using a Syber Green kit (FastStart DNA Masterplus Syber Green, Roche Diagnostic Co., Indianapolis, IN, USA), as previously described [7]. The results were expressed as relative amounts of the target gene using GAPDH as the inner reference gene, by means of relative quantification.

Data analysis

Statistical analysis was performed using SAS 9.3 (Cary, NC, USA). Data were first examined for normality using the Kolmogov-Smirnov test. Since the torque force and MicroCT data achieved normality, parametric methods were used for the comparisons. Then, One-Way Analysis of Variance (ANOVA) was used for comparison of the biomechanical retention of titanium implants evaluation and MicroCT parameters. When there were significant differences by ANOVA, a pair-wise comparison was performed by the Tukey test. Considering that the gene expression data did not achieved normality, non-parametric methods were used for the comparisons. Thus, the significance of differences in the relative gene expression levels analysis was compared using Kruskal-Wallis test. When there were significant differences by the Kruskal-Wallis test, a pair-wise comparison was performed by the Dunn's test. The significance level established for all analyses was 5%.

Results

Torque force evaluation

Data analysis revealed that resveratrol positively influenced biomechanical retention of titanium implants in diabetic animals considering that higher counter-torque values for implant removal were detected in the DM + RESV, DM + RESV + INS and Non-DM groups when compared with DM + PLAC group ($p < .05$). Figure 2 demonstrates the counter-torque force values observed in each experimental group.

MicroCT analysis

RESV therapy promoted positive effects in all evaluated parameters around the implants, providing reduced BIC, BV

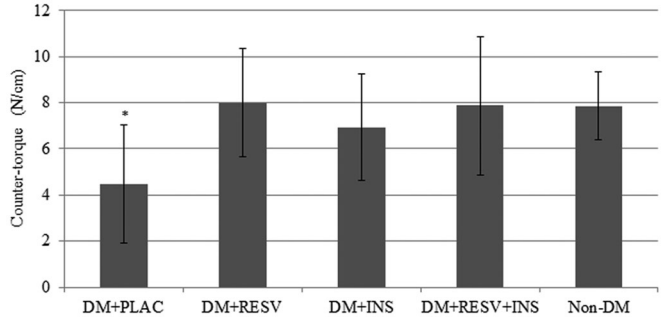

Figure 2. Graphic illustrating means and standard deviations of counter-torque values in all experimental groups. *Significant difference as compared to Non-DM, DM + RESV and DM + RESV + INS (Anova/Tukey; $p < .05$)

Table 1. Primer sequences for each gene, amplification profiles and the estimated length of qPCR product for each gene.

Gene	Sequence (5′–3′)	Length of qPCR product (bp)	Amplification profile [temperature (°C)/time (s)]
OPN	CCGGATGCAATCGATAGTG	164	95/10, 56/7, 72/8
BMP-2	GTCCCTACTGATGATGAGTTTCTC	170	95/10 56/8, 72/8
RANKL	AGCGCTTCTCAGGAGTT	156	95/5, 55/4, 72/6
OPG	GCAGAGAAGCACCTAGC	168	95/10, 56/8, 72/7
Osx	CCTCTTGCAACCAGGTC	150	95/10, 56/8, 72/7
β-catenin	ACTCTGAGAAACTTGTCCG	172	95/10, 56/8, 72/8
Dkk1	CGGGAATTACTGCAAAAACG	83	95/9, 59/9, 72/9
Runx2	GCCACTTACCACAGAGC	157	95/10, 56/8, 72/7
Lrp-5	GTGATAGCTGATGATCTGCC	163	95/10, 56/8, 72/7
GAPDH	TGAGTATGTCGTGGAGTCTACTG	159	95/10, 56/8, 72/7

Runt-Related Transcription Factor 2 (Runx2), Bone Morphogenetic Protein 2 (BMP-2), Osteopontin (OPN), Lipoprotein receptor-related protein 5 (Lrp-5), Osterix (Osx), Dickkopf 1 (Dkk1), Receptor activator of the NF-ĸB ligand (RANKL), osteoprotegerin (OPG), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

and Tb.Th values and higher PO and Tb.Sp in CSI + placebo group when compared to Non-CSI group ($p < .05$). The systemic intake of RESV, alone or in combination with INS, positively affected the percentage of BIC in diabetic rats, showing similar values when compared to non-diabetics ($p > .05$), whereas DM + PLAC group demonstrated reduced BIC levels when compared to these groups ($p < .05$). In addition, DM + RESV and Non-DM groups showed increased values of Tb.Th when compared to DM + PLAC group ($p < .05$). The RESV and/or INS therapy in diabetic animals not significantly influence BV/TV parameter ($p > .05$). Figure 3 illustrates all the microCT parameters evaluated in each group.

Gene expression levels

The gene expression analysis of this study revealed that the use of RESV, alone or associated with INS, downregulated the levels of RANKL, showing similar values when compared to non-DM animals ($p > .05$) and significantly reduced values when compared to DM + PLAC group ($p < .05$). Only the combined therapy of RESV and INS increased the levels of Runx2 when compared to DM + PLAC and DM + INS groups ($p < .05$). Higher levels of OPN, BMP-2, OPG and Runx2 and reduced levels of RANKL and Dkk-1 were detected in the Non-DM animals when compared to DM rats that received placebo ($p < .05$). Table 2 illustrates the gene expression outcomes.

Discussion

Considering the elevated global prevalence of DM and the augmented life expectancy of this individuals [1], the use of rehabilitations with dental implants in this patient profile probably will increase and investigations of predictable therapies to revert or contain the damaging effects of hyperglycaemia in the bone healing surrounding implants are

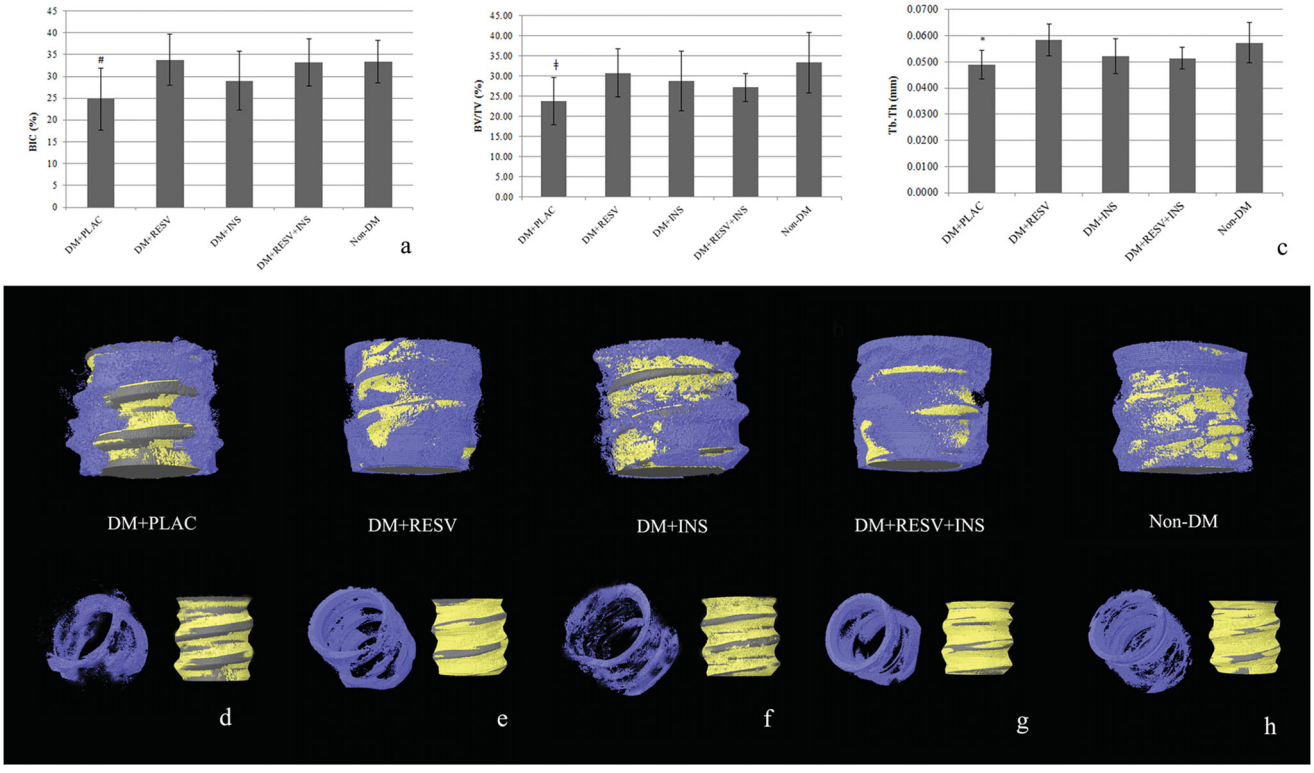

Figure 3. Graphics illustrating means and standard deviations of MicroCT analysis in all groups (a, b, c). Representative three-dimensional rendered images of the bone around implant (blue) and bone implant contact 'BIC' (yellow) at a controlled distance from the implant surface, in all experimental groups (d, e, f, g, h). +Significant difference when compared to Non-DM (Anova/Tukey; $p < .05$). *Significant difference when compared to DM + RESV and Non-DM (Anova/Tukey; $p < .05$). #Significant difference when compared to DM + RESV, DM + RESV + INS and Non-DM (Anova/Tukey; $p < .05$). Bone volume (BV); Trabecular Thickness (Tb.Th); Bone-implant contact (BIC).

Table 2. Mean ± (SD) of relative levels of mRNA for all genes (mRNA gene/mRNA GAPDH).

Gene/GAPDH	DM + PLAC	DM + INS	DM + RESV	DM + RESV + INS	Non-DM
OPN	0.226 (± 0.378) a	0.908 (± 0.906) ab	1.689 (± 2.966) ab	1.726 (± 2.738) ab	4.409 (± 3.904) b
BMP-2 ($\times 10^3$)	1.568 (± 1.055) a	7.540 (± 8.172) ab	10.560 (± 8.114) ab	8.139 (± 5.395) ab	14.299 (± 9.426) b
RANKL ($\times 10^3$)	4.407 (±3.233) a	0.671 (± 1.055) b	0.594 (± 0.629) b	0.442 (± 0.421) b	0.413 (± 0.289) b
OPG($\times 10^3$)	2.946 (±2.719) a	5.942 (± 6.009) ab	7.309 (± 9.619) ab	6.313 (± 3.370) ab	16.639 (± 9.263) b
Osx ($\times 10^3$)	2.781 (± 2.224) a	3.316 (± 3.090) a	2.196 (± 2.304) a	2.291 (± 3.493) a	8.865 (± 7.821) a
β-catenina ($\times 10^1$)	1.036 (± 1.206) a	1.110 (± 1.263) a	0.991 (± 0.915) a	3.002 (± 5.923) a	5.647 (± 10.558) a
Dkk1 ($\times 10^3$)	74.466 (±56.983) b	17.693 (±13.692) ab	11.488 (±7.256) ab	6.956 (±5.256) ab	9.409 (±6.562) a
Runx2 ($\times 10^1$)	0.489 (± 0.769) b	0.462 (± 0.465) b	2.918 (± 3.809) ab	3.199 (± 2.594) a	4.075 (± 3.325) a
Lrp-5 ($\times 10^3$)	0.630 (± 0.651) a	0.631 (± 0.783) a	1.085 (± 1.836) a	1.078 (± 1.911) a	1.582 (± 1.910) a

Different letters mean statistical difference (Kruskal-Wallis/Dunn; $p < .05$).

crucial. Taking into account the promising effects of RESV on bone formation [8,17,18,20,21], this study evaluated, for the first time, the influence of systemic intake of this natural polyphenolic substance in reversing the negative impact of hyperglycaemia on bone healing around titanium implants. In general, it was demonstrated that the daily use of RESV, associated or not with INS therapy, improved the peri-implant bone repair in diabetic conditions, while the combination of RESV with INS is necessary for the upregulation of Runx2 gene expression.

The biological impact of RESV on the bone formation and in suppressing the bone resorption has been confirmed in diverse *in vitro* and experimental studies [16,21,33,34]. In addition, recent investigations have demonstrated the positive influence of RESV on bone health under diabetic circumstances [8,28]. According to Gan et al. [35], RESV may improve the neovascularization capacity of bone marrow mononuclear cells from diabetic mice by *ex vivo* pre-treatment with this natural polyphenolic compound. Pino et al. [8] showed that the daily intake of RESV optimized the bone repair of critical-sized calvarial defects in animals with induced DM. In line, in a double-blind randomized-controlled trial, Bo et al. [28] revealed that supplementation with 500 mg resveratrol avoided bone density loss in type 2 diabetic patients [28]. Nevertheless, the present investigation is the first to reveal the role of RESV-therapy on bone healing around implants in diabetic conditions.

According to our data, the systemic treatment with RESV in diabetic animals, regardless INS combination, promoted elevated reverse-torque values for implant removal when compared to those treated with placebo ($p < .05$). In agreement with the counter-torque outcomes, MicroCT analyses of this study showed that RESV-therapy, alone or in association with INS, improved the BIC levels in diabetic rats, whereas DM animals treated with placebo achieved the lowest percentage of BIC ($p < .05$). Additionally, MicroCT analyses showed augmented Tb.Th levels in diabetic animals treated with RESV when compared to those that received placebo ($p < .05$). These encouraging results observed in this experiment highlight the benefits of using resveratrol as a therapeutic agent to ameliorate peri-implant bone healing, even under more challenging conditions for bone repair, as the presence of diabetes mellitus. Of interest, and in line with our results, previous data have already showed that the intake of RESV was also efficient to improve the osseous repair around titanium implants in systemically-healthy animals [20] and recent findings supported the positive role of this natural agent on the peri-implant bone healing in the presence of chronic cigarette smoke [22].

Although the molecular mechanisms modulated by RESV on bone metabolism are complex and remain not completely understood, there are evidences supporting that this plant agent encourages osteoblast differentiation from mesenchymal stem cells with relevant stimulatory pathway in osteoblast activity, besides to decrease the establishment of bone-resorbing osteoclasts [23,24]. The hopeful outcomes verified in the present study concerning the biomechanical retention of titanium implants and the MicroCT outcomes revealing the improved peri-implant bone repair obtained by RESV-treatment under hyperglycaemic conditions may be, partially, clarified by the capacity of this polyphenol in modulating osteogenic and osteoclastogenic mechanisms related to bone remodelling during bone healing processes.

In this context, the gene expression analysis of peri-implant bone surrounding implants of this experiment revealed that the daily intake of RESV in DM animals, in combination or not to insulin administration, promoted downregulation of osteoclastogenic mediator RANKL to normal levels, with significative reduction when compared to samples from DM animals treated with placebo ($p < .05$). It is well known that RANKL is a crucial molecule that acts as an accelerator of bone turnover, besides to stimulates the bone resorption during osseous remodelling, while OPG strongly prevents the RANK–RANKL interaction, avoiding osseous breakdown [36,37]. Innumerous researches have emphasized that DM increases the expression of RANKL and RANKL/OPG ratios in different experimental models, stimulating osteoclast recruitment and activity and augmenting bone resorption [7,15,38]. This data was supported by our study demonstrating that the mRNA levels of RANKL and OPG were, respectively, upregulated and downregulated in diabetic rats managed with placebo solution when compared to control-animals that were not submitted to DM induction ($p < .05$). In accordance with the data from the current investigation, the systemic therapy with RESV can reverse the harmful influence of diabetes around peri-implant tissues, decreasing the mRNA expression of RANKL in the bone biopsies during peri-implant repair phase. The promising influence of RESV-therapy in modulating the RANKL levels in osseous tissues was previously described in different biological circumstances related to bone turnover [32,37,38]. Recently, Khera et al. [25] reported that RESV restored RANKL/OPG ratio in the femur of rats submitted to osteoporosis model. In line, Franck et al. [26] verified that the systemic administration of this natural agent decreased the expression of RANKL/OPG in smoking rats, optimising the repair of critical-sized calvarial defects in animals submitted to this at-risk condition to bone repair. Shakibaei et al. [23] also showed that RESV has anti-osteoclastogenic properties that are intermediated probably through the suppression of RANKL expression, corroborating with our results, even in the presence of diabetes.

Importantly, the gene expression analyses of this study showed that only the combined therapy of RESV and INS in diabetic animals was efficient to increase the mRNA levels of Runx2, essential for osteoblastogenesis, in the biopsies of peri-implant bone tissue when compared to diabetic groups treated with placebo or insulin alone ($p < .05$). Runx2 is a key transcription factor that regulates the differentiation of the osteoblast linage [39]. Our outcomes showed that the presence of DM negatively modulated the gene expression of Runx2, reducing the levels of this bone-related biomarker in diabetic rats treated with placebo when compared to controls non-DM ($p < .05$), as previously demonstrated in other investigations that supported that the damage on bone formation in diabetic conditions may be related, among other

pathways, to the decreased expression of Runx2 [8,40]. Remarkedly, earlier data pointed-out up to 40% of reduction in mRNA levels of Runx2 in bone tissue of diabetic animals, fifteen days following the induction of STZ disease, when compared to the gene expression in control groups not submitted to DM [41].

Interestingly, the current study revealed that diabetic rats treated with daily RESV in association with INS displayed higher levels of Runx2, restoring the gene expression patter of this crucial osteoblastogenic factor, which may has contributed to the improved peri-implant bone repair observed in this experimental group in our biomechanical and MicroCT analyses. Earlier investigations have already indicated the that the insulin administration in diabetic conditions is associated with the upregulation of Runx2 [41]. The potential of RESV used alone in positively modulating the Runx2 expression on bone tissues has also reported in previous *in vitro* and experimental studies, however, without the presence of diabetes [23,24]. Considering the potential of RESV and INS used individually in the encouraging modulation of Runx2, it may be hypothesized that the synergistic actions of resveratrol and insulin potentialised, in the present study, the upregulation of gene expression of this molecule in the peri-implant bone biopsies of diabetic animals, supporting that in diabetic conditions the combined therapy of resveratrol and insulin are required to achieve modulation in the mRNA levels of Runx2.

Noteworthy, concerning the molecular impact of DM induction on peri-implant bone tissues, our results showed that DM treated with placebo presented downregulation of OPN, BMP-2, OPG and Runx2 and upregulation of RANKL and Dkk-1 when compared to Non-DM animals. These data confirm that DM promotes deleterious effects on bone tissue, although other osteoclast/blastogenesis pathways not investigated in this experiment may be modulated in the presence of this systemic condition. Interestingly, in this study, RANKL was positively modulated by RESV therapy and Runx2 expression was favoured by the association of RESV and insulin, validating the promising effect of RESV therapy in reversing the harmful impact of DM on peri-implant bone samples. Gene expression of other molecules, not addressed in this investigation, such as alkaline phosphatase, osteocalcin and collagen type I, for example, was revealed to be decreased in the bone tissue of DM animals two weeks following disease induction as compared to the mRNA amounts in the healthy control animals [41]. Thus, additional investigations could be relevant to determine other molecular pathways that can be modulated by the action of RESV in the presence of DM.

Recently, Serrão et al. [42] confirmed in an experimental study with diabetic rats that although the therapy with hypoglycemiant agents, as metformin, provides some molecular benefits in the osseointegration of implants (augmented OPG and declined RANKL/OPG levels in the medullary area), this therapeutic approach was not sufficient to positively modulate the harmful effect of hyperglycaemia on peri-implant bone healing at histometric levels. Previous studies have investigated other alternative therapies to overcome the damaging impact of hyperglycaemia in the healing of bone surrounding implants, including implant surface modification, parathyroid hormone therapy, mesenchymal stem cell management, hyperbaric oxygen treatment and doxycycline therapy [9–11]. Nevertheless, most of these therapies have presented not predictable outcomes on osseointegration of implants and have not been recognized as therapeutic alternatives to revert or contain the negative impact of DM on peri-implant bone healing, besides to be, in some cases, related to side effects for using on diabetic conditions.

Thus, considering the optimistic results detected following resveratrol treatment in the present investigation, its use could be considered, hereafter, to reverse the damaging impact of diabetes mellitus on peri-implant bone repair and therefore decrease the clinical complications around implants in this patient profile. Nevertheless, further researches, including clinical trials, should be developed to confirm the findings from this study, especially considering that this is the first one, to the authors knowledge, to evaluating the impact of resveratrol therapy in the reduction of the harmful effect of diabetes on peri-implant bone repair.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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