Original Research
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Does resveratrol favor peri-implant bone repair in rats with ovariectomy-induced osteoporosis? Gene expression, counter-torque and micro-CT analysis

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Abstract: This study investigated the influence of resveratrol on peri-implant repair and its effects on bone-related markers in ovariectomy-induced osteoporosis in rats. Animals were divided into: OVX+PLAC (n = 10): ovariectomized animals treated with placebo; OVX+RESV (n = 10): OVX treated with resveratrol; OVX+PLAC+ZOL (n = 10): OVX treated with PLAC and zoledronate; OVX+RESV+ZOL (n = 10): OVX treated with RESV and ZOL; and SHOVX+PLAC (n = 10): sham ovariectomy treated with PLAC. RESV and PLAC were administrated after ovariectomy and ZOL after six weeks after OVX, until the end of experiment. One implant was inserted in each tibiae of animals 18 weeks after ovariectomy. After 4 weeks, one implant was removed for counter-torque, and peri-implant tissue was collected for mRNA quantification of several osteogenic markers by PCR. The other tibia was submitted to micro-computed tomography analysis. Reduced counter-torque values, bone-implant contact (BIC) and bone volume fraction (BV/TV), and higher bone porosity (BP) were detected in OVX+PLAC group when compared to SHOVX+PLAC ($p < 0.05$). OVX+RESV rats presented lower BIC, BV/TV, and trabecular number (Tb.N), and augmented BP and trabecular spacing (Tb.Sp) when compared to SHOVX+PLAC ($p < 0.05$). Higher Tb.N and connectivity density (Conn.Dn) and reduced Tb.Sp were observed in OVX rats treated with ZOL, independently of RESV, when compared to OVX+PLAC and OVX+RESV groups ($p < 0.05$), whereas the combination ZOL+RESV promoted lower BP when compared to OVT+PLAC and OVX+RESV ($p < 0.05$). Gene expression was not influenced by RESV ($p > 0.05$), whereas ZOL promoted up-regulation of BMP-2 ($p<0.05$). RESV did not improve peri-implant bone repair in rats with ovariectomy-induced osteoporosis.

Keywords: Resveratrol; Plants, Medicinal; Dental Implants; Osseointegration; Osteoporosis; Gene Expression.

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Introduction

Osteopenia/osteoporosis are skeletal disorders that reduce bone volume and alter bone mineral density promoting negative effects on bone quality and deterioration of bone microarchitecture. Osteopenia/osteoporosis phenotype has been related to a higher risk for bone fragility and delayed bone repair.[1] Previous studies have shown that ovariectomized rats are characterized by an osteoporotic phenotype, with a slower healing of long bone fractures and a delayed tissue repair of tooth sockets after extractions compared to non-ovariectomized rats.[1,2]

Although systematic reviews of clinical studies have concluded that peri-implant bone healing was not influenced by osteoporotic-like conditions,[3] other findings support that bone loss around dental implants was more pronounced in osteoporotic individuals.[4,5] As limitation, several clinical studies fail to report whether patients were consistently using anti-osteoporotic drugs, such as bisphosphonates, which may influence the pattern of bone remodeling and the predictability of dental implant therapy in these individuals.[6]

Among the recommended antiresorptive drugs, bisphosphonates are the most frequently used, and evidence demonstrated that the use of these drugs is efficient for conserving bone density.[7] Nevertheless, the long-term use of bisphosphonates may provide undesirable effects, including osteonecrosis of the jaw, atrial fibrillation, bone and joint pain, atypical femoral fractures, and esophageal diseases, which have increased the controversy over the broad clinical use of these medications.[8]

Thus, the study of alternatives to conventional antiosteoporotic medications with minimal side effects is relevant. In addition, the interest regarding the therapeutic impact of natural agents on bone repair in osteoporotic circumstances has increased.[9,10]

Among the active substances derived from plants/food with important biological functions, resveratrol (trans-3,4,5'-trihydroxystilbene) (RESV) is a relevant agent able to improve bone formation and promote inhibitory impact on osteoclastogenesis.[11,12,13,14] Casarin et al.[12] revealed that resveratrol therapy improved the repair of critical-size bone defects and the biomechanical retention of implants, up-regulating the gene expression of essential osteogenic biomarkers. Promising outcomes were also described in diabetic conditions, showing that RESV positively influenced the repair of critical bone defects in calvaria of animals with induced diabetes mellitus.[13] Additionally, it was demonstrated that RESV reverses the harmful impact of smoking in the peri-implant repair, improving the modulation of bone-related markers.[14] Noteworthy, RESV has been suggested as a promising agent to alleviate the progression of osteoporosis.[15] Nevertheless, the impact of this natural substance on the peri-implant bone repair under osteoporotic conditions is not yet known.

The elderly population has progressively increased over the last decade and the combination of osteoporosis, periodontitis, and fewer teeth is a significant complication related to aging.[16] Thus, dental rehabilitations with osseointegrated implants in people with osteoporosis are important therapeutic alternatives to guarantee satisfactory masticatory function and adequate nutrition, besides improving the quality of life of this population, crucial aspects especially in elderly people.[17]

Based on the and considering the absence of information related to the impact of RESV on bone repair around implants under osteoporotic conditions, this investigation was designed to determine, for the first time, the influence of RESV-therapy on peri-implant repair using microcomputed tomography (micro-CT) and counter-torque analysis in ovariectomy-induced osteoporosis in rats. In addition, gene expression of bone-related markers was evaluated to better understand the molecular pathways associated with RESV impact on peri-implant healing.

Methodology

Animals

This experimental study was approved by the Animal Care and Use Committee of the university (Permit Number: 094/16) and performed according to the ARRIVE guidelines. The animal cohort was

composed of 50 16-week-old female Wistar rats,[18] weighing 348 ± 87 g at the beginning of the study. The animals were acclimatized for 15 days before experiments in the Bioterium of the university. Animals were maintained in temperature-controlled cages, exposed to a 12-h light-dark cycle, with food (Labina; Purina 1, Paulinia, Brazil) and water ad libitum.

Treatment groups

Animals were assigned to five experimental groups: ovariectomized animals treated with placebo (OVX+PLAC; n = 10); ovariectomized animals treated with resveratrol (OVX+RESV; n=10); ovariectomized animals treated with placebo and zoledronate (OVX+PLAC+ZOL; n = 10); ovariectomized animals treated with resveratrol and zoledronate (OVX+RESV+ZOL; n = 10); and animals submitted to sham ovariectomy surgery treated with placebo (SHOVX+PLAC; n = 10).

Sample size calculation

To calculate the sample size, previous data from investigations that evaluated the effect of natural compounds on the osseointegration in at-risk systemic conditions for peri-implant repair were used.[14,19,20] These studies used similar methodology for bone-implant contact (BIC) measurements, the primary outcome variable of this study, based on micro-CT analysis. The sample size was calculated using $\alpha = 0.05$ and 80% power. For the variability (σ = SD), a value of 5% was used. The minimum clinically significant value (δ) considered was 9%. It was determined that a minimum sample of 8 animals per group would be needed. However, considering that some animals may be lost during follow-up, the number of rats enrolled per group in this study was 10.

Study design

Study design is presented in Figure 1. Ovariectomy was performed at day zero. The administration of resveratrol (10 mg/Kg) and placebo was performed via gavage during all experiment period[21] after ovariectomy surgery. A stock solution of resveratrol (R5010-500MG, Sigma-Aldrich Ltda., São Paulo, 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. Zoledronic acid (SML-0223, Sigma-aldrich, St. Louis, USA) (1 mg/Kg), dissolved in phosphate-buffered saline (PBS), was administered after six weeks following ovariectomy until the end of the experiment by intraperitoneal injection, twice a week.[22] Placebo solution for zoledronic acid, composed of the same quantities of PBS as utilized in the preparation of zoledronic acid, was used. Implant surgery was performed 18 weeks following ovariectomy and animals were euthanized 4 weeks after implant placement.

Experimental induction of osteoporosis

After anesthesia, ovariectomy surgery was performed according to Shuai et al.[23] Animals of the non-ovariectomized group underwent sham operation as a control group (Sham).

Implant placement

Two titanium implants were inserted in each animal, one in each tibia. After anesthesia, 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, Brazil), was inserted in each tibiae until the screw threads had been totally fixed in the bone cortex[12]. The soft tissues were repositioned and sutured.

Post-operative period

The animals were examined regularly during the experiment for possible clinical or toxicological symptoms. Four weeks following the implant placement, the animals were euthanized by CO_2 inhalation. Then, the tibiae were dissected to access the implants: one of the implants was removed for torque force assessment and, subsequently, the peri-implant tissue was stored in RNAlater (Ambion Inc., Austin, USA) for gene expression analysis. The

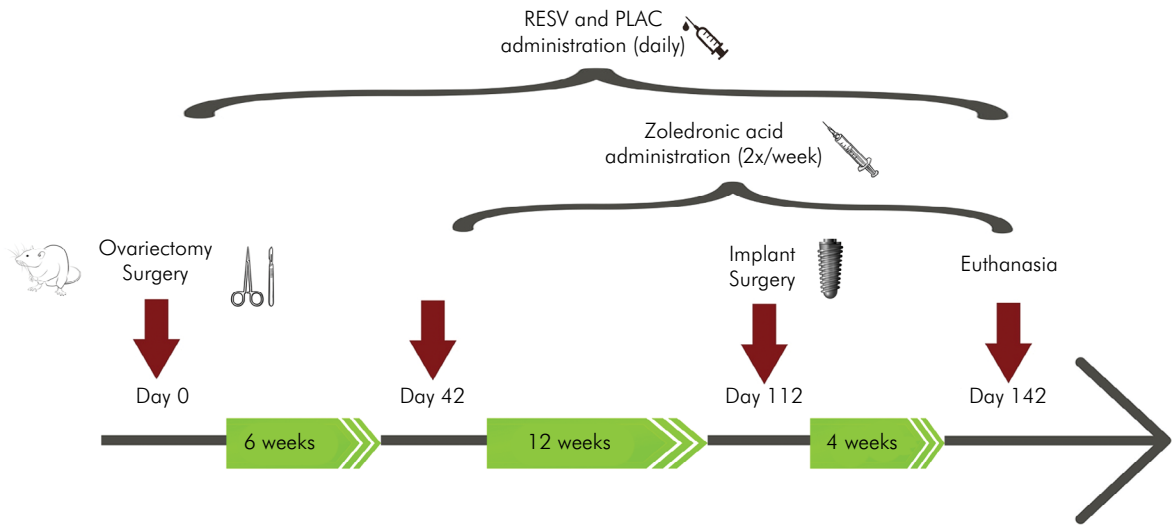


Figure 1. Study design diagram.

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 done using a torque meter 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 in the implant head to apply torque in the reverse direction of implant placement until rupture of the bone-implant interface was signaled by rotation of the implant. The torque force value achieved was considered as the torque required for the interruption of osseointegration.[14]

Microcomputed tomography analysis of implants

Non-demineralized specimens were scanned by a cone-beam 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 X 6 X 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 trans-axial direction, the volume of interest (VOI) was then defined, including the bone tissue surrounding the implant threads, as previously described.[14] Volumetric analyzes were done with CT-Analyzer® software (CT-Analyzer®, version 1.13.5.1+, Bruker, Kontich, Belgium). In each image, in the vertical direction, 214 standardized slices equivalent to the extension between the first and last threads present in the implant body were selected. In the horizontal direction, it was delimited the entire region occupied by the implant and that between the apex of the implant threads and the implant body. In order to evaluate trabecular bone tissue in each VOI, a gray scale (0–255) was used. The following parameters were assessed: a) bone-implant contact (BIC): percentage of bone tissue in contact with implant surface; b) bone volume fraction (BV/TV): percentage of VOI filled with bone tissue; c) bone porosity (BP): percentage of porosities present in the bone tissue determined in the VOI; d) trabecular number (Tb.N): number (%) of the bone trabeculae present in the VOI; e) trabecular spacing (Tb.Sp): total of spaces (mm) between the bone trabeculae present in the VOI; and f) connectivity density (Conn.Dn): density (mm^3) between the bone trabeculae present in the VOI.[14,24] BV/TV and BP indicates the portion of

mineralized bone tissue, and Conn.Dn indicates the degree of trabecular branching, while Tb.Sp and Tb.N reproduce the organization and amount of trabeculae. BIC reflects the ratio of implant surface in direct contact with bone. 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 for gene expression assays were stored in RNALater solution at -70°C for mRNA levels quantification of the molecules by real-time polymerase chain reaction (qRT-PCR): bone morphogenetic protein-2 (BMP-2), osteoprotegerin (OPG), receptor activator of NF-KB ligand (RANKL), and runt-related transcription factor-2 (Runx2). Total RNA from the biopsies was isolated by the Trizol method according to the manufacturer's recommendation. RNA samples were resuspended in diethylpyrocarbonate-treated water and stored at −70 °C. The RNA concentration was determined from the optical density using a micro-volume spectrophotometer. Total RNA was DNase treated (Turbo DNA-frees, Ambion Inc., Austin, TX, USA), and 1 µg was used for complementary DNA (cDNA) synthesis. The reaction was carried out using the First-Strand cDNA synthesis kit (Roche Diagnostic Co., Indianapolis, IN, USA), following the manufacturer's recommendations.

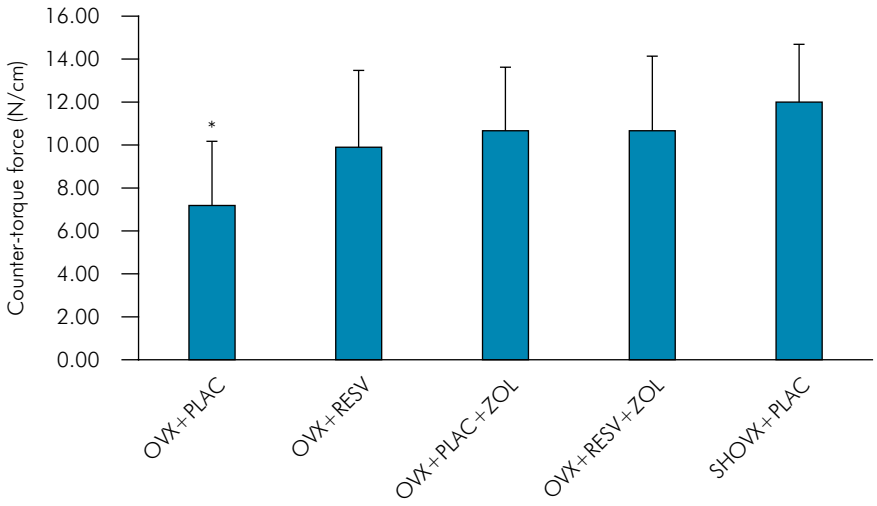
Primers were designed using probe-design software (Light-Cycler Roche probe design software, Diagnostics GmbH, Mannheim, Germany). The amplification profiles, primer sequences, and lengths of PCR products are shown in Table. The qPCR reactions were performed in a real-time PCR apparatus (LightCycler® 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[22]. 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 micro-CT 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 micro-CT 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, a pair-wise comparison was performed by the Dunn's test. The significance level established for all analyses was 5%.

Table. 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)]
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
Runx2	GCCACTTACCACAGAGC	157	95/10, 56/8, 72/7
GAPDH	TGAGTATGTCGTGGAGTCTACTG	159	95/10, 56/8, 72/7



*Significant difference compared to SHOVX+PLAC (Anova/Tukey; p<0.05).

Figure 2. Means and standard deviations of counter-torque values in all experimental groups.

Results

Torque force evaluation

Data analysis revealed lower biomechanical retention of titanium implants in OVX+PLAC animals when compared to SHOVX+PLAC group (p <0 .05). No significant differences were observed among the other groups (p > 0.05). Figure 2 demonstrates the counter-torque force values observed in each experimental group.

Micro-CT analysis

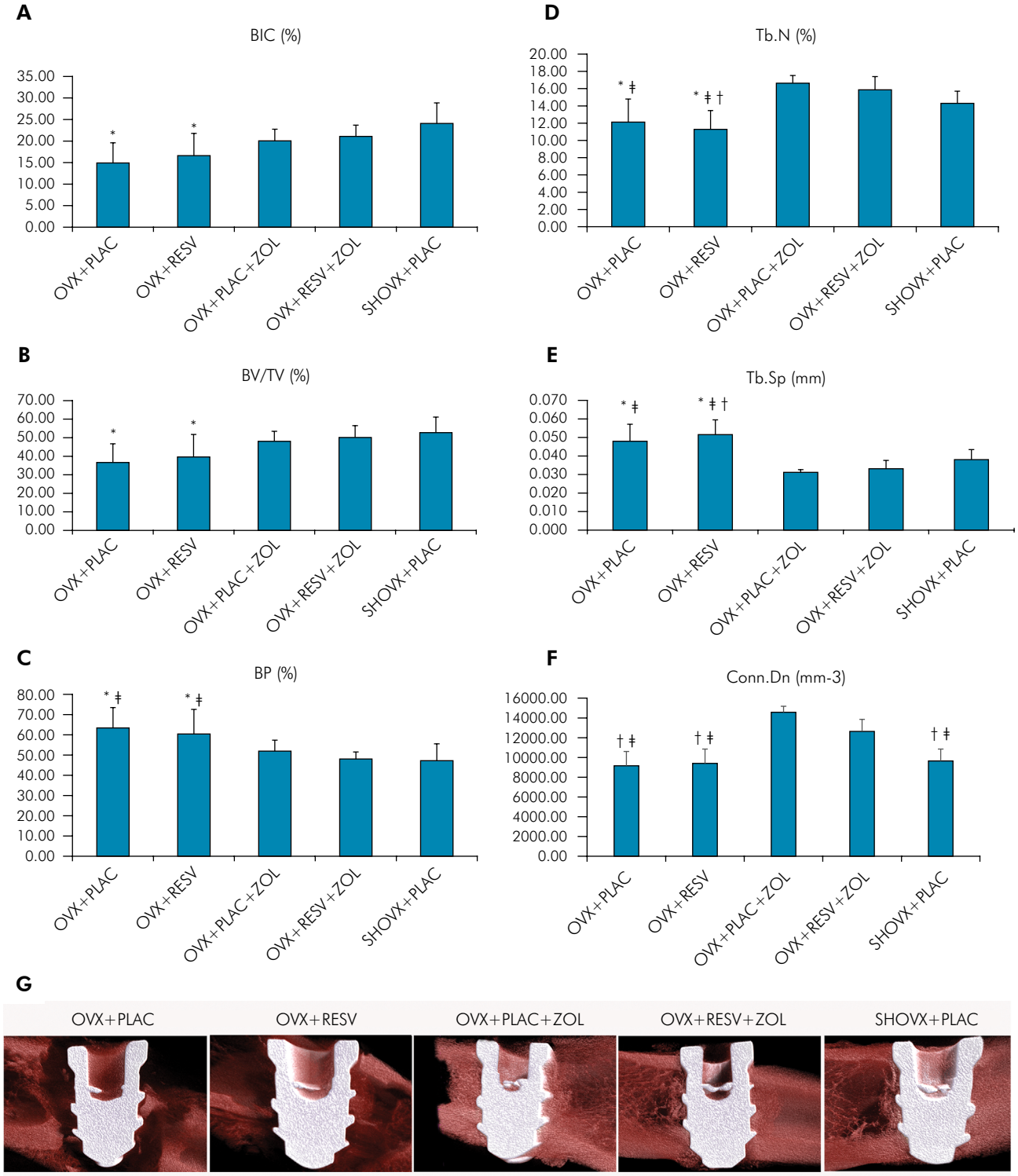
Ovariectomy promoted negative effects around the implants, providing reduced BIC and BV/TV and higher BP in OVX+PLAC group when compared to SHOVX+PLAC (p < 0.05). In general, the intake of RESV alone in ovariectomized rats was not able to optimize the peri-implant bone repair, since no differences were detected when compared to OVX+PLAC group (p > 0.05). Both OVX+PLAC and OVX+RESV presented lower BIC, BV/TV, and Tb.N percentages and increased values of BP and Tb.Sp when compared to SHOVX+PLAC group (p < 0.05). Higher levels of Tb.N and Conn.Dn and reduced values of Tb.Sp were observed in ovariectomized animals treated with ZOL, independently of RESV intake, when compared to OVX+PLAC and OVX+RESV groups (p < 0.05), whereas the combined use of ZOL and RESV promoted lower BP levels when compared to OVT+PLAC and OVX+RESV groups (p < 0.05). Figure 3 demonstrates the micro-CT outcomes.

Gene expression levels

Significant up-regulation of RANKL mRNA levels was found in OVX+PLAC group when compared to SHOVX+PLAC animals (p < 0.05). Down-regulation of BMP-2 levels was detected in OVX+PLAC and OVX+RESV groups when compared to OVX+PLAC+ZOL, OVX+RESV+ZOL, and SHOVX+PLAC animals (p < 0.05). No differences were detected among groups in the levels of OPG, Runx-2, and RANKL/OPG (p > 0.05). Figure 4 illustrates the gene expression outcomes.

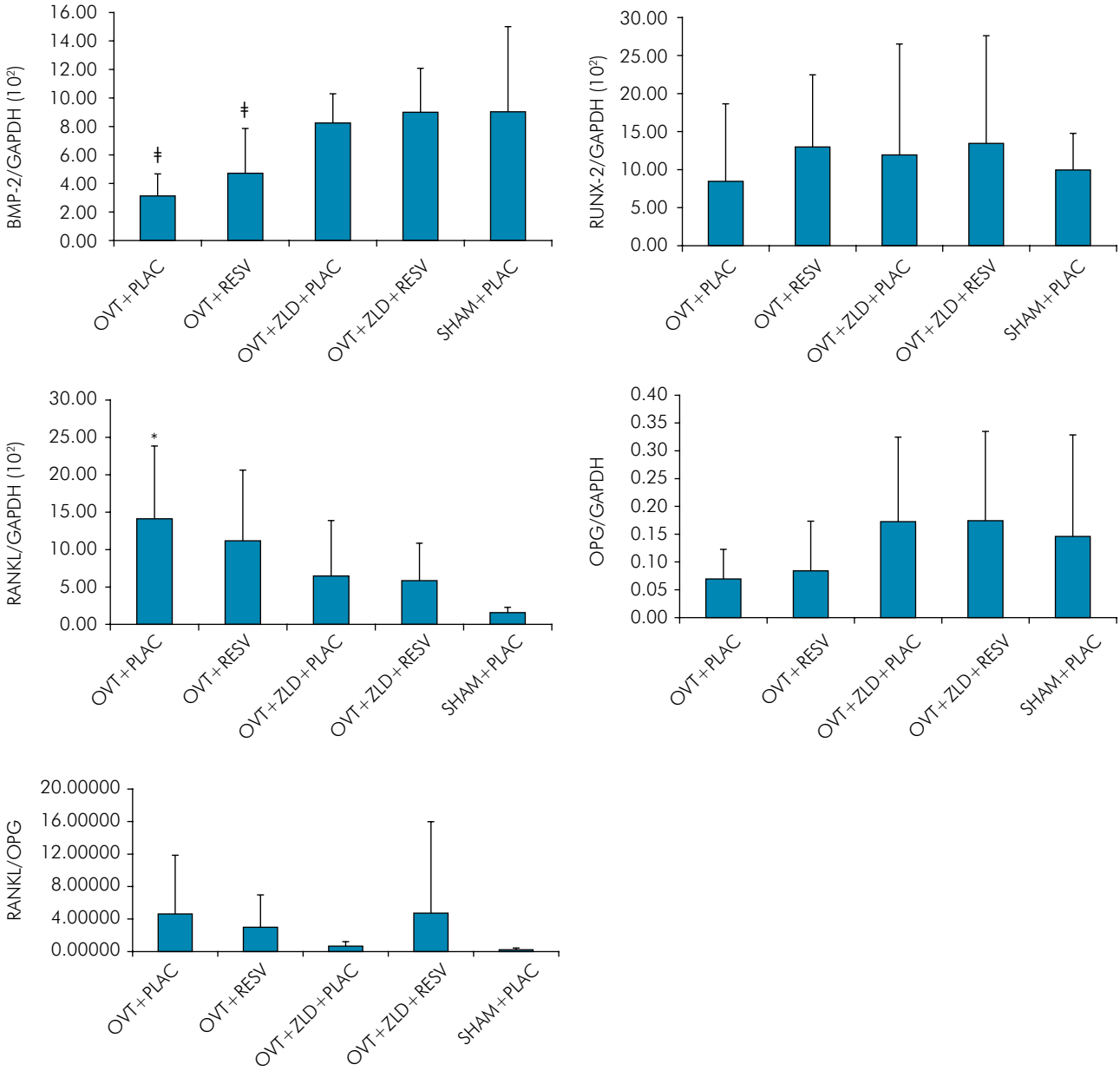
Discussion

Since the global prevalence of osteoporosis has increased and considering the higher life expectancy of word population, rehabilitations with osseointegrated implants is becoming more necessary, particularly considering the elevated occurrence of periodontitis and successive tooth loss in osteoporotic individuals.[16] Considering the favorable proprieties of RESV on bone metabolism,[11,12,13,14] this investigation studied, for the first time, the impact of daily systemic use of this polyphenol in reversing the negative



BIC: bone-implant contact; BV/TV: bone volume fraction; BP: bone porosity; Tb.N: trabecular number; Tb.Sp: trabecular spacing; Conn. Dn: connectivity density; *Significant difference compared to SHOVX+PLAC (Anova/Tukey; $p<0.05$); ‡Significant difference compared to OVX+RESV+ZOL (ANOVA/Tukey, $p<0.05$); †Significant difference compared to OVX+PLAC+ZOL (ANOVA/Tukey, $p<0.05$).

Figure 3. Means and standard deviations of micro-CT analysis of all groups (A-E). Representative three-dimensional rendered images of bone repair around the implant in all experimental groups (G).



*Significant difference compared to SHOVX+PLAC (Kruskal-Wallis; $p<0.05$); ǂ Significant difference compared to OVX+PLAC+ZOL, OVX+RESV+ZOL, and SHOVX+PLAC (Kruskal-Wallis, $p<0.05$).

Figure 4. Means and standard deviations of gene expression analysis.

impact of osteoporosis on bone healing around titanium implants. In general, it was verified that the use of RESV, associated or not with ZOL therapy, did not improve the peri-implant bone repair in osteoporotic conditions.

In this study, ovariectomy promoted negative effects around the implants, providing reduced bone-implant contact and bone volume and higher porosity in OVX+PLAC group, besides compromised implants biomechanical performance, when compared to SHOVX+PLAC ($p < 0.05$). In agreement with our data, previous experimental studies have demonstrated compromised implants biomechanical performance in osteoporotic-like conditions[25]. Accordantly, clinical data have also highlighted that peri-implant bone loss is more noticeable in people with osteoporosis

and that osteoporosis may contribute to delayed healing time around implants, and even implant failure, especially in augmented alveolar bone.[5,26]

Bisphosphonates, as zoledronate, are the most used drug to treat the consequences on bone tissue promoted by osteoporosis. This medication acts primarily by inhibiting osteoclast-mediated bone resorption and regularizing the high proportion of bone turnover.[27] The relationship between bisphosphonates-therapy and development of osteonecrosis of jaw has been reported[27], as well as the benefit of bisphosphonates intake on peri-implant bone repair under osteoporotic state.[27] In addition, micro-CT analyses from the current investigation revealed that higher amount of trabeculae and degree of trabecular branching were detected in ovariectomized animals treated with ZOL, independently of RESV-therapy, as well as reduced spaces between trabeculae, when compared to ovariectomized animals treated with placebo or resveratrol ($p < 0.05$). Contrary to the results of micro-CT analyses, the counter-torque data from the present investigation did not reveal an impact of zoledronate on biomechanical retention of titanium implants ($p > 0.05$). It is important to highlight that whereas counter-torque analysis determines the force required to rupture the bone-implant interface, micro-CT technique provides a non-destructive method to study per-implant samples and the option of performing three dimensional investigations on a virtual representation of a sample, determining BIC values and the quality of bone formation based on additional parameters, as evaluated in this study. Thus, these are methodologies with different forms of analysis, which can produce variation in results, as observed in the outcomes of this experimental investigation.

Concerning the impact of resveratrol on bone repair around implants in rats with ovariectomy-induced osteoporosis, micro-CT analyses showed that RESV therapy in ovariectomized rats was not able to optimize the peri-implant bone repair, as it did not improve bone-implant contact, proportion of mineralized bone tissue, and amount of trabeculae when compared to OVX+PLAC animals ($p > 0.05$), besides presenting higher bone porosity and amount of spaces between trabeculae when compared to SHOVX+PLAC ($p < 0.05$). Despite this finding, previous findings have already demonstrated that the therapy with resveratrol was effective to optimize the peri-implant bone repair in healthy conditions.[12] More recently, other researchers reported the positive impact of this polyphenol in the presence of chronic cigarette smoke[14] and the efficiency of RESV in reversing the negative effect of alcohol on bone repair around implants in senile female rats.[28] Importantly, data from another study indicated that the use of RESV on the repair of critical bone defects in calvaria of animals with induced diabetes mellitus positively influenced bone repair, regardless of the presence of insulin.[13]

The mechanisms attributed to RESV to up-regulate bone formation and reduce bone resorption has been supported by various researches.[11,12,13,14] Interestingly, numerous studies have established the promising impact of RESV on bone tissues under osteoporotic circumstances.[15,29] Khera et al.[29] showed that RESV restores the RANKL/OPG ratio in the femur of rat osteoporosis model. *In vitro* experiments demonstrated that the suppressed osteogenic differentiation of BMSCs undergoing TNF-α induction is enhanced by RESV therapy, which may alleviate the advance of osteoporosis.[15] However, to our knowledge, the current investigation is the first to report the effect of RESV treatment on peri-implant bone healing in osteoporotic conditions.

Osteoporosis is a grave skeletal disorder characterized by an important decrease in bone mineral density, with deterioration of microarchitecture of bone tissues.[1] In addition, OVX induces oxidative stress and simultaneously promotes up-regulation of RANKL levels, elevated production of TRAP-5b, and harmful impact on bone microstructure.[30] Conversely, RESV therapy in ovariectomized rats may reverse these variations *in vivo*, providing antioxidant effects, decreasing RANKL and TRAP-5b levels, and suppressing bone microarchitecture breakdown,[31] consistent with earlier findings.[14,24,29] Moreover, it was recently demonstrated that RESV reversed the negative impact of smoking on peri-implant repair in the tibia of rats by down-regulating RANKL/OPG and Lrp-5 levels, which are related to oscleoclastogenenic pathways.[14]

Although RESV promotes positive effects on bone metabolism both in *in vivo* experiments and in molecular assays,[11,12,13,14,29] gene expression data and micro-CT analyses from the present study support that the harmful impact of osteoporosis on bone tissues around implants probably outweighs the benefits of this natural agent. Thus, this therapeutic approach, as administrated in this study, was not sufficient to positively modulate the destructive impact of osteoporosis on peri-implant bone healing. It could be hypothesized that in the presence of osteoporosis, the use of higher doses of resveratrol could be effective. Noteworthy, Zhao et al.[31] demonstrated that the effects of RESV on bone mineral density and trabecular microarchitecture was dose-dependent in ovariectomized rats, preventing OVX-induced decrease of bone mineral density in the proximal tibia and femoral neck with daily RESV doses of 40 and 80 mg/kg. In the current study, the dose used was 10 mg/kg, as utilized previously in other researches with promising influence on bone repair.[11,12,13,14] Interestingly, Zhao et al.[31] reported that only higher doses of resveratrol (40 and 80 mg/kg) stimulated the binding of RANKL to RANK to decrease osteoclastic activity, inhibiting bone resorption. Thus, although the period of RESV therapy in the current study was longer than that employed in previous studies,[11,12,13,14,31] further research using different RESV doses and administration protocols could be pertinent to determine if this polyphenol would provide additional benefits on bone healing around implants in the presence of osteoporosis.

It is important to mention that two of the experimental groups of the current research were treated with a well-established drug usually employed in osteoporotic disorders, zoledronate. In agreement with the promising outcomes provided by ZOL-therapy on micro-CT parameters, the gene expression analysis of peri-implant bone surrounding implants of this experiment revealed that the administration of zoledronate in ovariectomized animals, independently of RESV intake, positively modulated the gene expression of BMP-2. In line with our findings, Im et al.[32] showed a significantly increased osteoblastic cell number and enhanced gene expression of BMP-2, besides the up-regulation of type I collagen and osteocalcin with alendronate and risedronate in cell cultures. Although the beneficial effects of bisphosphonates on osteoblast proliferation, maturation, and differentiation has been previously evidenced,[32] other different pathways may be signalized to provide the anabolic influence of this drug on osteoblasts and the inhibitory effects on osteoclasts, distinct from the molecules evaluated in this study. However, even considering all these well supported signaling pathways associated with the positive effects of bisphosphonates under osteoporotic bone tissues, the potential risk of bisphosphonates-related osteonecrosis of the maxillary and mandibular bones in osteoporotic patients undergoing dental implant therapy cannot be disregarded, especially depending on the route of administration and duration of medication therapy.[33]

Taking into account that the number of patients with senile or postmenopausal osteoporosis requiring implant treatment has increased with the growth of the elderly population,[16] the interest in alternative therapeutic approaches to reversing the harmful effects of osteoporosis on peri-implant bone loss, without the risk of relevant adverse effects, is relevant.

Although in the present investigation no signs of inflammation or bone necrosis were observed, it is well established that bisphosphonates may promote innumerous undesirable effects, including osteonecrosis of jaw,[8,27] highlighting the need for alternative therapeutic approaches. Recently, Movahedian Attar et al.[34] demonstrated the protective role of resveratrol against osteonecrosis after tooth extraction in rats treated with bisphosphonates. Importantly, it has been described that bisphosphonates promote anti-angiogenesis effects, besides inducing overproduction of reactive oxygen species, which cause damage to cell DNA.[35,36] Evidence has demonstrated that resveratrol has antioxidant effects and may prevent DNA damage by eliminating free radicals and promoting the activities of antioxidant enzymes.[35,36] Additionally, it is relevant to emphasize that resveratrol promotes pro-angiogenic effects during the tissue healing.[37] Thus, it could be hypothesized that the antioxidant and pro-angiogenic actions of resveratrol could positively interfere in eventual osteonecrosis

occurrences. Furthermore, *in vitro* findings showed that resveratrol significantly increased osteoblast proliferation and differentiation in cells treated with bisphosphonates, suggesting that the use of resveratrol could be promising in the clinical management or prevention of bisphosphonate-related osteonecrosis of the jaws.[38] However, further investigations are required to support these data.

Another important topic to be mentioned is that, in the current study, estrogen replacement therapy was not adopted as treatment. One of the objectives of this study was to propose an alternative therapy to avoid substances recognized for promoting side effects in the presence of osteoporosis, as previously stated. Several adverse effects may be caused by estrogen replacement, including vascular events and breast carcinoma.[39] In addition, evidence demonstrated that estrogen replacement therapy in postmenopausal women did not promote significant impact on the reduction of dental implant failure rate.[40] Interestingly, recent data of a systematic review showed that hormone replacement therapy promoted a slightly significant harmful effect regarding implant survival and marginal bone loss around implants.[41]

In conclusion, this study demonstrated that resveratrol may not favor peri-implant bone repair in rats with ovariectomy-induced osteoporosis. Additional investigations should be developed to confirm the findings from this study, especially considering that this is the first one, to the authors knowledge, to determine the impact of resveratrol therapy in containing the damaging effect of osteoporosis on peri-implant bone repair.

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References

1. Cheung WH, Miclau T, Chow SK, Yang FF, Alt V. Fracture healing in osteoporotic bone. Injury. 2016 Jun;47 Suppl 2:S21-6. https://doi.org/10.1016/S0020-1383(16)47004-X
2. Chen CH, Wang L, Serdar Tulu U, Arioka M, Moghim MM, Salmon B, et al. An osteopenic/osteoporotic phenotype delays alveolar bone repair. Bone. 2018 Jul;112:212-9. https://doi.org/10.1016/j.bone.2018.04.019
3. Medeiros FC, Kudo GA, Leme BG, Saraiva PP, Verri FR, Honório HM, et al. Dental implants in patients with osteoporosis: a systematic review with meta-analysis. Int J Oral Maxillofac Surg. 2018 Apr;47(4):480-91. https://doi.org/10.1016/j.ijom.2017.05.021
4. Holahan CM, Koka S, Kennel KA, Weaver AL, Assad DA, Regennitter FJ, et al. Effect of osteoporotic status on the survival of titanium dental implants. Int J Oral Maxillofac Implants. 2008 Sep-Oct;23(5):905-10.
5. Giro G, Chambrone L, Goldstein A, Rodrigues JA, Zenóbio E, Feres M, et al. Impact of osteoporosis in dental implants: A systematic review. World J Orthop. 2015 Mar;6(2):311-5. https://doi.org/10.5312/wjo.v6.i2.311
6. Zandi M, Dehghan A, Amini P, Rezaeian L, Doulati S. Evaluation of mandibular fracture healing in rats under zoledronate therapy: A histologic study. Injury. 2017 Dec;48(12):2683-7. https://doi.org/10.1016/j.injury.2017.10.026
7. Reginster JY. Antifracture efficacy of currently available therapies for postmenopausal osteoporosis. Drugs. 2011 Jan;71(1):65-78. https://doi.org/10.2165/11587570-000000000-00000
8. Kos M. Association of dental and periodontal status with bisphosphonate-related osteonecrosis of the jaws: a retrospective case controlled study. Arch Med Sci. 2014 Feb;10(1):117-23. https://doi.org/10.5114/aoms.2014.40738
9. Zhu B, Xue F, Zhang C, Li G. Ginkgolide B promotes osteoblast differentiation via activation of canonical Wnt signalling and alleviates osteoporosis through a bone anabolic way. J Cell Mol Med. 2019 Aug;23(8):5782-93. https://doi.org/10.1111/jcmm.14503
10. Bei J, Zhang X, Wu J, Hu Z, Xu B, Lin S, et al. Ginsenoside Rb1 does not halt osteoporotic bone loss in ovariectomized rats. PLoS One. 2018 Sep;13(9):e0202885. https://doi.org/10.1371/journal.pone.0202885
11. Tseng PC, Hou SM, Chen RJ, Peng HW, Hsieh CF, Kuo ML, et al. Resveratrol promotes osteogenesis of human mesenchymal stem cells by upregulating RUNX2 gene expression via the SIRT1/FOXO3A axis. J Bone Miner Res. 2011 Oct;26(10):2552-63. https://doi.org/10.1002/jbmr.460

12. Casarin RC, Casati MZ, Pimentel SP, Cirano FR, Algayer M, Pires PR, et al. Resveratrol improves bone repair by modulation of bone morphogenetic proteins and osteopontin gene expression in rats. Int J Oral Maxillofac Surg. 2014 Jul;43(7):900-6. https://doi.org/10.1016/j.ijom.2014.01.009
13. Pino DS, Casarin RC, Pimentel SP, Cirano FR, Corrêa MG, Ribeiro FV. Effect of Resveratrol on Critical-Sized Calvarial Defects of Diabetic Rats: Histometric and Gene Expression Analysis. J Oral Maxillofac Surg. 2017 Dec;75(12):2561.e1-10. https://doi.org/10.1016/j.joms.2017.07.167
14. Ribeiro FV, Pimentel SP, Corrêa MG, Bortoli JP, Messora MR, Casati MZ. Resveratrol reverses the negative effect of smoking on peri-implant repair in the tibia of rats. Clin Oral Implants Res. 2019 Jan;30(1):1-10. https://doi.org/10.1111/clr.13384
15. Chen XH, Shi ZG, Lin HB, Wu F, Zheng F, Wu CF, et al. Resveratrol alleviates osteoporosis through improving the osteogenic differentiation of bone marrow mesenchymal stem cells. Eur Rev Med Pharmacol Sci. 2019 Jul;23(14):6352-9. https://doi.org/10.26355/eurrev_201907_18459
16. Renvert S, Persson RE, Persson GR. Tooth loss and periodontitis in older individuals: results from the Swedish National Study on Aging and Care. J Periodontol. 2013 Aug;84(8):1134-44. https://doi.org/10.1902/jop.2012.120378
17. Schimmel M, Srinivasan M, McKenna G, Müller F. Effect of advanced age and/or systemic medical conditions on dental implant survival: A systematic review and meta-analysis. Clin Oral Implants Res. 2018 Oct;29(S16 Suppl 16):311-30. https://doi.org/10.1111/clr.13288
18. Bonucci E, Ballanti P. Osteoporosis-bone remodeling and animal models. Toxicol Pathol. 2014 Aug;42(6):957-69. https://doi.org/10.1177/0192623313512428
19. Cirano FR, Pimentel SP, Casati MZ, Corrêa MG, Pino DS, Messora MR, et al. Effect of curcumin on bone tissue in the diabetic rat: repair of peri-implant and critical-sized defects. Int J Oral Maxillofac Surg. 2018 Nov;47(11):1495-503. https://doi.org/10.1016/j.ijom.2018.04.018
20. Corrêa MG, Ribeiro FV, Pimentel SP, Benatti BB, Silva PHF, Casati MZ, et al. 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. [Acta Odontol Scand. 2020 Jul 22. https://doi.org/10.1080/00016357.2020.1797159
21. Franck FC, Benatti BB, Andia DC, Cirano FR, Casarin RC, Corrêa MG, et al. Impact of resveratrol on bone repair in rats exposed to cigarette smoke inhalation: histomorphometric and bone-related gene expression analysis. Int J Oral Maxillofac Surg. 2018 Apr;47(4):541-8. https://doi.org/10.1016/j.ijom.2017.08.004
22. Kim JW, Tatad JC, Landayan ME, Kim SJ, Kim MR. Animal model for medication-related osteonecrosis of the jaw with precedent metabolic bone disease. Bone. 2015 Dec;81:442-8. https://doi.org/10.1016/j.bone.2015.08.012
23. Shuai B, Shen L, Yang Y, Ma C, Zhu R, Xu X. Assessment of the Impact of Zoledronic Acid on Ovariectomized Osteoporosis Model Using Micro-CT Scanning. PLoS One. 2015 Jul;10(7):e0132104. https://doi.org/10.1371/journal.pone.0132104
24. Vasconcelos KFF, Corpas LS, Silveira BM, Laperre K, Padovan LE, Jacobs R, et al. MicroCT assessment of bone microarchitecture in implant sites reconstructed with autogenous and xenogenous grafts: a pilot study. Clin Oral Implants Res. 2017 Mar;28(3):308-13. https://doi.org/10.1111/clr.12799
25. Yildiz A, Esen E, Kürkçü M, Damlar I, Dağlioğlu K, Akova T. Effect of zoledronic acid on osseointegration of titanium implants: an experimental study in an ovariectomized rabbit model. J Oral Maxillofac Surg. 2010 Mar;68(3):515-23. https://doi.org/10.1016/j.joms.2009.07.066
26. Erdoğan O, Shafer DM, Taxel P, Freilich MA. A review of the association between osteoporosis and alveolar ridge augmentation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Dec;104(6):738.e1-13. https://doi.org/10.1016/j.tripleo.2007.04.008
27. Ruggiero SL. Bisphosphonate-related osteonecrosis of the jaw: an overview. Ann N Y Acad Sci. 2011 Feb;1218(1):38-46. https://doi.org/10.1111/j.1749-6632.2010.05768.x
28. Tao ZS, Zhou WS, Yang M, Xu H. Resveratrol reverses the negative effect of alcohol on hydroxyapatite-coated implant osseointegration in senile female rats. Z Gerontol Geriatr. 2020 Octt;53(6):538-45.: https://doi.org/10.1007/s00391-019-01595-3
29. Khera A, Kanta P, Kalra J, Dumir DM. Resveratrol restores the level of key inflammatory cytokines and RANKL/OPG ratio in the femur of rat osteoporosis model. J Women Aging. 2019 Nov-Dec;31(6):540-52. https://doi.org/10.1080/08952841.2018.1522126
30. Feng YL, Jiang XT, Ma FF, Han J, Tang XL. Resveratrol prevents osteoporosis by upregulating FoxO1 transcriptional activity. Int J Mol Med. 2018 Jan;41(1):202-12. https://doi.org/10.3892/ijmm.2017.3208
31. Zhao H, Li X, Li N, Liu T, Liu J, Li Z, et al. Long-term resveratrol treatment prevents ovariectomy-induced osteopenia in rats without hyperplastic effects on the uterus. Br J Nutr. 2014 Mar;111(5):836-46. https://doi.org/10.1017/S0007114513003115
32. Im GI, Qureshi SA, Kenney J, Rubash HE, Shanbhag AS. Osteoblast proliferation and maturation by bisphosphonates. Biomaterials. 2004 Aug;25(18):4105-15. https://doi.org/10.1016/j.biomaterials.2003.11.024
33. Graves LL, Bukata SV, Aghazadehsanai N, Chang TI, Garrett NR, Friedlander AH. Patients receiving parenteral bisphosphonates for malignant disease and having developed an atypical femoral fracture are at risk of concomitant osteonecrosis of the jaw: an evidence-based review. J Oral Maxillofac Surg. 2016 Dec;74(12):2403-8. https://doi.org/10.1016/j.joms.2016.06.003

34. Movahedian Attar B, Razavi SM, Daneshmand M, Davoudi A. Protective effects of resveratrol against osteonecrosis at the extraction site in bisphosphonate-treated rats Int J Oral Maxillofac Surg. 2020 Nov;49(11):1518-22. https://doi.org/10.1016/j.ijom.2020.02.019
35. Sener G, Kapucu C, Cetinel S, Cikler E, Ayanoğlu-Dülger G. Gastroprotective effect of leukotriene receptor blocker montelukast in alendronat-induced lesions of the rat gastric mucosa. Prostaglandins Leukot Essent Fatty Acids. 2005 Jan;72(1):1-11. https://doi.org/10.1016/j.plefa.2004.04.005
36. Ohnuki H, Izumi K, Terada M, Saito T, Kato H, Suzuki A, et al. Zoledronic acid induces S-phase arrest via a DNA damage response in normal human oral keratinocytes. Arch Oral Biol. 2012 Jul;57(7):906-17. https://doi.org/10.1016/j.archoralbio.2011.11.015
37. Huang X, Sun J, Chen G, Niu C, Wang Y, Zhao C, et al. Resveratrol Promotes Diabetic Wound Healing via SIRT1-FOXO1-c-Myc Signaling Pathway-Mediated Angiogenesis. Front Pharmacol. 2019 Apr;10:421. https://doi.org/10.3389/fphar.2019.00421
38. Borsani E, Bonazza V, Buffoli B, Nocini PF, Albanese M, Zotti F, et al. Beneficial effects of concentrated growth factors and resveratrol on human osteoblasts *in vitro* treated with bisphosphonates. BioMed Res Int. 2018 May;2018:4597321. https://doi.org/10.1155/2018/4597321
39. Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, et al.; Women's Health Initiative Investigators. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA. 2003 Oct;290(13):1729-38. https://doi.org/10.1001/jama.290.13.1729
40. August M, Chung K, Chang Y, Glowacki J. Influence of estrogen status on endosseous implant osseointegration [published correction appears in J Oral Maxillofac Surg 2002 Jan;60(1):134]. J Oral Maxillofac Surg. 2001 Nov;59(11):1285-9. https://doi.org/10.1053/joms.2001.27515
41. Stavropoulos A, Bertl K, Pietschmann P, Pandis N, Schiødt M, Klinge B. The effect of antiresorptive drugs on implant therapy: systematic review and meta-analysis. Clin Oral Implants Res. 2018 Oct;29 Suppl 18:54-92. https://doi.org/10.1111/clr.13282