The Effects of Simvastatin on Bone Healing around Titanium Implants in Osteoporotic Rats

Zhibin Du[1], Jiang Chen[1], Fuhua Yan[1], Yin Xiao[2]

1 Department of Oral Implant, Affiliated Stomatological Hospital of Fujian Medical University, Fuzhou, China

2 Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia

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Corresponding author:

Jiang Chen

Department of Oral Implant, Affiliated Stomatological Hospital of Fujian Medical University, Fuzhou, China
Tel: 86-591-83754882
Fax: 86-591-83700838
Email: dentistjiang@hotmail.com

Fuhua Yan
Department of Periodontology, Affiliated Stomatological Hospital of Fujian Medical University, Fuzhou, China
Email: fhyan2005@126.com

Abstract

Objective: Osteoporosis is known to impair the process of implant osseointegration. The recent discovery that statins (HMG-CoA reductase inhibitors) act as bone anabolic agents, suggests statins can be used as potential agents in the treatment of osteoporosis. Therefore, we hypothesized that statins will promote osteogenesis around titanium implants in subjects with osteoporosis.

Materials and methods: Fifty-four female Sprague Dawley rats, aged 3 months old, were randomly divided into three groups: Sham-operated group (SHAM; n=18); ovariectomized group (OVX; n=18); and ovariectomized with Simvastatin treatment group (OVX+SIM; n=18). 56 days after being ovariectomized (OVX), screw-shaped titanium implants were inserted into the tibiae. Simvastatin was administered orally at 5mg/kg each day after the placement of the implant in the OVX+SIM group. The animals were sacrificed at either 28 or 84 days after implantation and the undecalcified tissue sections were obtained. Bone-to-implant contact (BIC) and bone area (BA) within the limits of implant threads were measured around the cortical (zone A) and cancellous (zone B) bone regions. Furthermore, bone density (BD) of zone B in a 500 μm -wide zone lateral to the implants was also measured.

Results: There were no significant differences in BIC and BA measurements in zone A in any of the three groups at either 28 or 84 days after implantation ($P>0.05$). By contrast, in zone B significant differences in the measurement of BIC, BA, and BD were observed at 28 and 84 days between all three groups. Bone healing decreased with lower BIC, BA and BD around implant in OVX group compared with other two groups and Simvastatin reversed the negative effect of OVX on bone healing around

implants with the improvement of BIC, BA, and BD in zone B.

Conclusion: Osteoporosis can significantly influence bone healing in the cancellous bone around titanium implants and Simvastatin was shown to significantly improve the osseointegration of pure titanium implants in osteoporotic rats.

INTRODUCTION

The successful outcome of dental implants depends on the firm osseointegration of implants which depends on there being sufficient bone tissue in the jawbone. There are various factors that may adversely affect the osseointegration of implants such as implant material, shape, and surface chemistry and patient variables such as bone quantity and quality (Puleo & Nanci 1999). Osteoporosis has been defined as a systemic skeletal disease characterized by gradual loss and microarchitectural deterioration of bone tissue which results in increased bone fragility and susceptibility to fracture (Wactawski-Wende 2001). Previous researchers have demonstrated that osteoporosis can impair the process of implant osseointegration (Motohashi, et al. 1999, Yamazaki, et al. 1999, Pan, et al. 2000, Lugero, et al. 2000, Duarte, et al. 2003, Duarte, et al. 2005) . Osteoporosis must therefore be considered one of the most important factors in the failure of implant osseointegration.

Various therapeutic approaches, seeking to improve osseointegration of implants with bone affected by osteoporosis, have been reported (Qi, et al. 2004). However, most drugs currently available for treatment of osteoporosis predominantly work by suppressing bone resorption, which slows down bone turnover resulting in reduced bone loss. In order to improve osseointegration of implants in osteoporotic bone, drugs capable of enhancing bone formation are therefore more relevant from a clinical perspective. The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, Simvastatin, is a widely used cholesterol-lowering drug and inhibits hepatic cholesterol biosynthesis. Recent studies have shown the beneficial effects of statins on

bone mineral density (Uzzan, et al. 2007, Serin-Kilicoglu & Erdemli, 2007). It has been suggested that several statin drugs, including Simvastatin, increase the mRNA expression of BMP-2 in osteoblasts, with a subsequent increase in bone formation, when injected subcutaneously over the murine calvaria (Mundy, et al. 1999). Several animal and human studies have been performed to elucidate the clinical importance of statins. Most experimental and epidemiological studies have shown statins to have beneficial effects on bone metabolism, as evaluated by bone mineral density (BMD) (Bauer, et al. 2004, Montagnani, et al. 2003, Rejnmark, et al. 2004) and fracture risk (Pasco, et al. 2002, van Staa,et al 2001, Skoglund, et al. 2002). Statins have therefore been proposed as potential agents in the treatment of osteoporosis. Previous studies also suggest that Simvastatin can promote osteogenesis around titanium implants (Ayukawa, et al. 2004). To our knowledge there are no studies that have investigated the effects of statins on bone healing around titanium implants in subjects with osteoporosis. In this study we evaluate, by histometric analysis, whether Simvastatin can promote bone healing around titanium implants in an osteoporosis rat model.

MATERIALS AND METHODS

Experimental Animals

Fifty-four 3 months old female Sprague-Dawley rats, weighing approximately 240-300g at the beginning of the experiments, were obtained from the animal resource centre (SLAC laboratory animal Co. Ltd, Shanghai, China). The rats were housed at the university animal house with controlled room temperature (22±2 ^{0}C)

and humidity (50±20%). The room light was controlled with a 12hr light - 12hr dark cycle. Commercial laboratory rat chow (Experimental Animal Centre of Zhejiang University, China) and water were available *ad libitum*. The study was conducted according to a protocol approved by the Animal Care and Use Committee of Fujian Medical University.

Experimental design

The rats were anesthetized by abdominal administration of 2.5% pentobarbital sodium (Chemical Agent Co, Shanghai, China) at 45mg/kg body weight. The animals were divided into three groups and each group contained 18 rats by randomized block design: Sham-operated group (SHAM) (n=18); ovariectomized group (OVX) (n=18); and ovariectomized with Simvastatin treatment group (OVX+SIM) (n=18). In the OVX and OVX+SIM groups the ovaries were exposed and completely excised bilaterally, using an abdominal approach (Geary & Asarian 2001). In the SHAM group the ovaries were exposed and equal volumes of fat tissue besides each ovary was excised. After surgery the fascia and skin were closed and sutured. Nine animals from each group were sacrificed either at 28 or 84 days after implantation.

Implantation and treatment

Screw-shaped implants made from commercially pure titanium were used in this study (Jiuzhou Co, Xian, China). Total length of each implant was 5mm, thread diameter was 2mm, and pitch 1mm. The implants were cleaned in absolute ethanol in an ultrasonic bath and sterilized by autoclaving. Osteoporotic changes due to

ovariectomy was verified by sacrificing two rats from each group and recovering the proximal tibial metaphyses and uterine horns 56 days after the operation. The specimens were fixed in 10% neutral buffered formalin. The tibiae were decalcified with 5% nitric acid, trimmed and embedded in paraffin. Cross sections of uterine horn were cut and 5μm thick sections longitudinal to the axis of tibiae were prepared and stained with haematoxylin-eosin (H&E) for light microscope examination.

56 days after ovariectomy surgery, screw shaped titanium implants were inserted in the left tibiae of each rat according to a method described in a previous study (Nociti et al. 2002). Briefly, under general anaesthesia, the surface of proximal metaphases of the tibiae was exposed by an incision approximately 10 mm in length. Under constant saline irrigation, bicortical implant beds were drilled with a dental burr (Zhongbang, Co, Xian, China) at a rotary speed not exceeding 1500 rpm and the implant was placed until the screw thread had been completely introduced into the bone cortex, after which the soft tissue was replaced and sutured. Simvastatin was administered orally at 5mg /kg per day according to the previous study (Mundy, et al. 1999) after implant surgery to the OVX+SIM group. Saline was given as a placebo to the other two groups.

Histometric procedure

After sacrificing the rats the left tibiae with the pure titanium implant were harvested and fixed in 4% neutral formalin for 48 hours. The specimens were dehydrated in a series of graded alcohol and embedded in polyester resin without decalcification. Undecalcified sections, approximately 50μm thick and longitudinal to

the implant, were cut with Letize 1600 saw microtome and bone grinding slice technique. Three sections were obtained from each tibia and stained with 0.1% toluidine blue and methylene blue-basic fuchsin solution separately for light microscopy. The percentage of bone-to-implant contact (BIC%) and bone area (BA%) from both sides of the implant in the cortical (zone A) and cancellous (zone B) areas, and bone density (BD%) in a 500 μm -wide zone lateral to the implants surface in zone B were measured bilaterally according previous methods (Duarte, et al. 2003, Nociti, et al. 2002). The data from three sections per specimen were averaged.

Statistical methods

The data of all rats were summarized, presenting mean ± standard deviation (SD). Statistical analyses were performed using the SigmaStat^{TM} statistics package (SPSS Inc., USA). A Wilk-Shapiro test for normality was conducted on all parameters and a one-way analysis of variance (ANOVA) was carried out on all groups followed by post hoc *Turkey's* test (alpha=0.05) to determine the difference between individual groups. In the Zone B group of BA (unequal variances (p=0.022) in parameters), the Kruskal-Wallis test (KW-test) was performed. If KW-test was significant, the Nemenyi's nonparametric multiple comparison was performed. Additionally, the multivariate analysis of variance (MANOVA) test was used to compare the effects of bone healing based on the parameters of BIC, BA, and BD between 28 days and 84 days of each relevant group.

RESULTS

Bone and uterine horns histological changes after ovariectomy

At day 56 after ovariectomy proximal tibial metaphyses showed significant osteoporotic changes in both the OVX and OVX+SIM groups. In the area of cancellous bone, the trabeculae were sparse, irregular and discontinuous. Significant atrophy was seen in the uterine horn after ovariectomy. The cavities were small and the glandular organs were scarce. By contrast, in the SHAM group the trabeculae were dense and arranged regularly in the tibia. The glandular organs of uterine horn were abundant with the normal size of uterine horn cavity (Fig. 1).

Light microscopy

Twenty-eight days after implantation, both SHAM and OVX+ SIM groups showed similar morphologies. The preexisting cancellous bone areas were connected and newly formed bone was seen around the implant surface forming a reticular structure. In the area of cortical bone most of the implant surface was in direct contact with the new woven bone. In the area of the medullary cavity most of the implant surface was covered with a thick new bone lamella, which was connected with preexisting bone by newly formed trabeculae at several locations. In some areas of the interface, between implants and new bone, a layer of early fibrous woven bone could be detected. Osteocytes were apparent in the newly formed bone matrix surrounding the implant. In the OVX groups preexisting cancellous bone around the implants was thin and sparse compared with the OVX+SIM and SHAM groups. The new bone

covering the implant surface was thin and disconnected (Fig. 2).

84 days after implantation histological results showed more newly formed trabeculae than at 28 days in both the SHAM and OVX+ SIM groups. The changes in both groups were similar in the cancellous and cortical bone areas. The newly formed bone attached to the implant surface had matured and become thicker compared to those at 28 days. At 84 days the amount of new bone on implant surface in all three groups was more than the new bone formation at 28 days respectively. In the OVX group the amount of new bone covering the implant surface was thin and disconnected compared to the new bone formation seen in the OVX+SIM and SHAM groups. However, in the cortical bone area, most of the implant surfaces were covered with mature lamella bone, and no significant differences were seen between the three groups (Fig. 3).

Histomorphometry

Intergroup comparisons showed that Simvastatin promoted bone healing around implants in cancellous bone, but had no effect on the bone healing around implants in the cortical bone area. In zone A (cortical bone area) intergroup analysis did not reveal any significant difference among the three groups in the measurement of BIC and BA (ANOVA test, $P>0.05$) at either 28 or 84 days after implantation. In zone B (cancellous bone area) the measurement of BIC, BA, and BD showed significantly difference among the three groups (for 28 days, $P=0.012$ (BIC), $P=0.005$ (BA, Kruskal-Wallis test), $P=0.003$ (BD) and for 84 days, $P=0.005$ (BIC) , $P=0.001$(BA),

P=0.002 (BD)). The BIC, BA, and BD showed higher volume in the SHAM and OVX+SIM groups than the OVX group (for 28 days, OVX+SIM *vs* OVX, P=0.012 (BIC), P=0.044 (BA, Nemenyi's test), P=0.018 (BD); SHAM *vs* OVX, P=0.007 (BIC), P=0.009 (BA, Nemenyi's test), P=0.001 (BD)and for 84 days, OVX+SIM *vs* OVX, P=0.023 (BIC), P=0.001 (BA), P=0.029(BD); SHAM *vs* OVX, P=0.002 (BIC), P=0.001 (BA), P=0.001 (BD)). All three groups showed better bone healing around implants in 84 days compared with 28 days (MANOVA test, alpha=0.05, F=56.806, P<0.001). However, no difference was observed between SHAM and OVX+SIM groups at either 28 or 84 days after implantation (Turkey's test, P>0.05). (Tables 1 and 2).

DISCUSSION

The 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, Simvastatin, is a widely used cholesterol-lowering drug, which inhibits cholesterol biosynthesis. Statins lower cholesterol synthesis by the inhibition of the mevalonate pathway. Recently, it has been reported that the liposoluble statin, Simvastatin, could increase the expression of bone morphogenetic protein (BMP)-2 mRNA in osteoblasts and, as a result, promote bone formation (Mundy, et al. 1999). The anabolic effect of statins on bone metabolism have further been reported in a number of animal studies (Skoglund, et al. 2002, Oxlund, et al. 2001, Pytlik, et al. 2003). This phenomenon has generated great interest amongst researchers to investigate potential applications of statins in the treatment of some bone related diseases, as well as implantology.

Despite this level of interest, there are no reports, to our knowledge, that evaluate the potential role of statins on the bone quality around titanium implants placed in osteoporotic subjects, although a limited number of studies have reported the effects of statins on the osseointegration of implants (Ayukawa, et al. 2004).

In this study, we investigated the potential application of Simvastatin to improve osseointegration of titanium implants in rats with ovariectomy induced osteoporosis. This osteoporosis model has frequently been used to study postmenopausal osteoporosis patients undergoing oral implantation in a clinical setting (Motohashi, et al. 1999, Pan, et al. 2000, Duarte, et al. 2003, Duarte, et al. 2005, Qi, et al. 2004).

56 days after having undergone ovariectomy, the histological changes of proximal tibial metaphysis and uterine horns confirmed that the ovaries in the rats of OVX and OVX+SIM groups had been completely excised and osteoporosis successfully induced.

It is widely accepted that bone quality is an important factor governing the success of titanium implants. The negative effects of osteoporosis on implant osseointegration have been reported in several preclinical studies conducted in OVX animals (Motohashi, et al. 1999, Yamazaki, et al. 1999, Pan, et al. 2000, Lugero, et al. 2000, Duarte, et al. 2003, Duarte, et al. 2005). In our study, both 28 and 84 days after implantation, the BIC and BD in zone A and zone B, and BA in zone B were significantly decreased in OVX group compared with SHAM group (Tables 1 and 2). Similar observations in previous studies also showed decreased BIC, BD, and BA in osteoporotic rats (Duarte, et al. 2003). These results suggests that osseointegration of implants may be compromised

in patients with primary osteopenia, osteoporosis, or secondary osteoporosis (for example, due to rheumatoid arthritis and/or prolonged corticosteroid treatment).

In our study we demonstrated that the administration of 5mg/kg/day of Simvastatin induce a significant increase in BIC, BD, and BA in zone B at 28 and 84 days in OVX rats. Moreover, we observed no difference between the OVX+SIM groups and SHAM groups (Tables 1 and 2). These results suggest that Simvastatin can effectively overcome the negative impact osteoporosis exert on the osseointegration of titanium implants. Simvastatin appears to promote bone healing around titanium implants by increasing implant-bone contact rate and cancellous bone volume, thus obtaining secondary stability.

The enhanced bone formation around implant, induced by Simvastatin in osteoporotic rats, may be associated with an increased expression of the bone morphogenic protein 2, which stimulates osteoblast differentiation (Mundy, et al. 1999). Statin drugs are known to enhance the expression of VEGF, a bone anabolic factor, in osteoblasts (Maeda, et al. 2003) and to regulate osteoblast function by increasing the expression of bone sialoprotein (BSP), osteocalcin (OCN), and type I collagen (COL1), as well as suppressing the gene expression of collagenases such as MMP-1 and MMP-13 (Maeda, et al. 2004). In addition, by competitive inhibition of HMG-CoA reductase, statins interfere with the malevonate pathway, leading to decreased protein prenylation, necessary for normal osteoclast function (Mundy, et al. 1999). The action of the former would have an anabolic effect on bone and the latter would have an antiresorptive effect, similar to the biphosphonates that act downstream in the

malevonate pathway.

In summary, the present study has observed, histologically, that Simvastatin influences bone healing around titanium implants in osteoporotic rats, which supports the anabolic effect of Simvastatin on bone metabolism in OVX rats. The results suggest a potential application of Simvastatin in dental implantation for osteoporosis patient. However, more studies are needed, by investigating whether Simvastatin plays a similar role in humans.

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Legends:

Figure 1. Comparison of the changes of uterus and proximal tibia in SHAM (A and C) and OVX (B and D) groups at 56 days after surgery. The size of uterus and the uterus cavity were normal at SHAM rats with abundant glandular organ in comparison to the OVX rat, which showed significantly decrease in the size of uterus and uterus cavity (H&E stain with 4x magnification). The regularity and connectivity of trabecular were disrupted at the OVX rats (D) compared to the SHAM rats (C) (Hematoxylin and Eosin stain with 4x magnification). Bar = 2mm.

Figure 2. Histological observations after 28 days of implantation in proximal tibia. The new bone formation on implant surface in SHAM (A and D) and OVX+SIM (C and F) groups showed similar changes. Most of the implant surface was in direct contact with the new woven bone and newly formed trabeculae around implants were connected with preexisting bone. The new bone formation around implant surface in OVX group (B and E) was thin and discontinuous. (A, B, and C magnification 2x, bar = 2mm; D, E, and F magnification 10x, bar = 0.5mm).

Figure 3. Histological observations after 84 days of implantation in proximal tibia. The new bone around implant surface in SHAM (A and D) and OVX+SIM (C and F) groups was significantly thicker than the new bone formed on implant surface in OVX group (B and E). The amount of new bone on implant surface after 84 days in all three groups was more than the new bone at 28 days respectively. (A, B, and C magnification 2x, bar=2mm; D, E, and F magnification 10x, bar=0.5mm).

Table 1 Bone histomorphometric indices at 28 days after implantation ($\bar{x}$±SD，%)

Groups	BIC		BA		BD
	Zone A	Zone B$^{\Delta}$	Zone A	Zone B$^{\Delta}$	Zone B$^{\Delta}$
SHAM	70.48±6.86	58.00±11.95	60.09±18.71	31.81±11.82	25.10±9.19
OVX	72.94±4.92	34.92±12.63$^{*\#}$	68.52±17.99	14.45±4.44$^{*\#}$	9.81±4.18$^{*\#}$
OVX + SIM	72.50±6.69	56.06±17.31	72.41±12.45	27.03±8.06	19.63±7.01

Δ: P=0.012 (BIC), 0.005 (BA, Kruskal-wallis test), 0.003 (BD)

*: P=0.007 (BIC), 0.009 (BA, Nemenyi' test), 0.001 (BD) *vs:* SHAM group

#: P=0.012 (BIC), 0.044 (BA, Nemenyi' test), 0.018 (BD) *vs:* OVX+SIM group.

Data are means ± SD.

Table 2 Bone histomorphometric indices at 84 days after implantation($\bar{x}$±SD ,%)

Groups	BIC		BA		BD
	Zone A	Zone B$^{\Delta}$	Zone A	Zone B$^{\Delta}$	Zone B$^{\Delta}$
SHAM	86.88±5.60	79.47±12.06	84.72±4.74	51.61±9.42	31.74±10.29
OVX	79.48±6.26	55.84±15.20$^{*\#}$	85.56±6.66	25.03±7.56$^{*\#}$	15.72±5.05$^{*\#}$
OVX + SIM	83.38±6.49	71.78±7.26	83.38±5.98	41.66±6.54	24.67±4.32

Δ: P=0.005 (BIC), 0.001 (BA), 0.002 (BD)

*: P=0.002 (BIC), 0.001 (BA), 0.029 (BD) *vs:* SHAM group

#: P=0.001 (BIC), P=0.001 (BA), P=0.029 (BD) *vs:* OVX+SIM group.

Data are means ± SD.

REFERENCES

Ayukawa, Y., Okamura, A. & Koyano, K. (2004) Simvastatin promotes osteogenesis around titanium implants. *Clinical OralIimplants Research* ***15****: 346-350.*

Bauer, D.C., Mundy, G.R., Jamal, S.A., Black, D.M., Cauley, J.A., Ensrud, K.E., van der Klift, M. & Pols, H.A. (2004) Use of statins and fracture: Results of 4 prospective studies and cumulative meta-analysis of observational studies and controlled trials. *Archives of Internal Medicine* ***164****: 146-152.*

Duarte, P.M., Cesar Neto, J.B., Goncalves, P.F., Sallum, E.A. & Nociti, F.H. (2003) Estrogen deficiency affects bone healing around titanium implants: A histometric study in rats. *Implant Dentistry* ***12****: 340-346.*

Duarte, P.M., Goncalves, P.F., Casati, M.Z., Sallum, E.A. & Nociti, F.H., Jr. (2005) Age-related and surgically induced estrogen deficiencies may differently affect bone around titanium implants in rats. *Journal of Periodontology* ***76****: 1496-1501.*

Geary, N. & Asarian, L. (2001) Estradiol increases glucagon's satiating potency in ovariectomized rats. *American Journal of Physiology* ***281****: R1290-1294.*

Lugero, G.G., de Falco Caparbo, V., Guzzo, M.L., Konig, B., Jr. & Jorgetti, V. (2000) Histomorphometric evaluation of titanium implants in osteoporotic rabbits. *Implant Dentistry* ***9****: 303-309.*

Maeda, T., Kawane, T. & Horiuchi, N. (2003) Statins augment vascular endothelial growth factor expression in osteoblastic cells via inhibition of protein prenylation. *Endocrinology* ***144****: 681-692.*

Maeda, T., Matsunuma, A., Kurahashi, I., Yanagawa, T., Yoshida, H. & Horiuchi, N. (2004) Induction of osteoblast differentiation indices by statins in mc3t3-e1 cells. *Journal of Cellular Biochemistry* ***92:*** *458-471.*

Montagnani, A., Gonnelli, S., Cepollaro, C., Pacini, S., Campagna, M.S., Franci, M.B., Lucani, B. & Gennari, C. (2003) Effect of simvastatin treatment on bone mineral density and bone turnover in hypercholesterolemic postmenopausal women: A 1-year longitudinal study. *Bone* ***32:*** *427-433.*

Motohashi, M., Shirota, T., Tokugawa, Y., Ohno, K., Michi, K. & Yamaguchi, A. (1999) Bone reactions around hydroxyapatite-coated implants in ovariectomized rats. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* ***87:*** *145-152.*

Mundy, G., Garrett, R., Harris, S., Chan, J., Chen, D., Rossini, G., Boyce, B., Zhao, M. & Gutierrez, G. (1999) Stimulation of bone formation in vitro and in rodents by statins. *Science* ***286:*** *1946-1949.*

Nociti, F.H., Jr., Cesar, N.J., Carvalho, M.D. & Sallum, E.A. (2002) Bone density around titanium implants may be influenced by intermittent cigarette smoke inhalation: A histometric study in rats. *The International Journal of Oral & Maxillofacial Implants* ***17:*** *347-352.*

Oxlund, H., Dalstra, M. & Andreassen, T.T. (2001) Statin given perorally to adult rats increases cancellous bone mass and compressive strength. *Calacified Tissue International* ***69:*** *299-304.*

Pan, J., Shirota, T., Ohno, K. & Michi, K. (2000) Effect of ovariectomy on bone remodeling adjacent to hydroxyapatite-coated implants in the tibia of mature rats. *Journal of Oral and Maxillofacial Surgery* ***58:*** *877-882.*

Pasco, J.A., Kotowicz, M.A., Henry, M.J., Sanders, K.M. & Nicholson, G.C. (2002) Statin use, bone mineral density, and fracture risk: Geelong osteoporosis study. *Archives of Internal Medicine* ***162:*** *537-540.*

Puleo, D.A. & Nanci, A. (1999) Understanding and controlling the bone-implant interface. *Biomaterials* ***20:*** *2311-2321.*

Pytlik, M., Janiec, W., Misiarz-Myrta, M. & Gubala, I. (2003) Effects of simvastatin on the development of osteopenia caused by ovariectomy in rats. *Polish Journal of Pharmacology* ***55:*** *63-71.*

Qi, M.C., Zhou, X.Q., Hu, J., Du, Z.J., Yang, J.H., Liu, M. & Li, X.M. (2004) Oestrogen replacement therapy promotes bone healing around dental implants in osteoporotic rats. *International Journal of Oral and Maxillofacial Surgery* ***33:*** *279-285.*

Rejnmark, L., Buus, N.H., Vestergaard, P., Heickendorff, L., Andreasen, F., Larsen, M.L. & Mosekilde, L. (2004) Effects of simvastatin on bone turnover and bmd: A 1-year randomized controlled trial in postmenopausal osteopenic women. *Journal of Bone and Mineral Research* ***19:*** *737-744.*

Serin-Kilicoglu, S. & Erdemli, E. (2007) New addition to the statin's effect. *The Journal of Trauma* ***63:*** *187-191.*

Skoglund, B., Forslund, C. & Aspenberg, P. (2002) Simvastatin improves fracture healing in mice. *Journal of Bone and Mineral Research* ***17:*** *2004-2008.*

Uzzan, B., Cohen, R., Nicolas, P., Cucherat, M. & Perret, G.Y. (2007) Effects of statins on bone mineral density: a meta-analysis of clinical studies. *Bone* ***40:*** *1581-1587.*

van Staa, T.P., Wegman, S., de Vries, F., Leufkens, B. & Cooper, C. (2001) Use of statins and risk of fractures. *Journal of the American Medical Association* ***285:*** *1850-1855.*

Wactawski-Wende, J. (2001) Periodontal diseases and osteoporosis: Association and mechanisms. *Annals of Periodontology* ***6:*** *197-208.*

Yamazaki, M., Shirota, T., Tokugawa, Y., Motohashi, M., Ohno, K., Michi, K. & Yamaguchi, A. (1999) Bone reactions to titanium screw implants in ovariectomized animals. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* ***87****: 411-418.*