Acta Biomaterialia 6 (2010) 1610–1618

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Local application of fluvastatin improves peri-implant bone quantity and mechanical properties: A rodent study

Yasuko Moriyama [a,*], Yasunori Ayukawa [a], Yoichiro Ogino [a], Ikiru Atsuta [a], Mitsugu Todo [b], Yoshihiro Takao [b], Kiyoshi Koyano [a]

[a] Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan
[b] Research Institute for Applied Mechanics, Kyushu University, Fukuoka, Japan

ARTICLE INFO

Article history:
Received 11 March 2009
Received in revised form 15 October 2009
Accepted 28 October 2009
Available online 1 November 2009

Keywords:
Bone healing
Dental implant
Drug release
Osseointegration
Mechanical test

ABSTRACT

Statins are known to stimulate osteoblast activity and bone formation. This study examines whether local application of fluvastatin enhances osteogenesis around titanium implants in vivo. Ten-week-old rats received a vehicle gel (propylene glycol alginate (PGA)) or PGA containing fluvastatin (3, 15, 75 or 300 μg) in their tibiae just before insertion of the implants. For both histological and histomorphometric evaluations undecalcified ground sections were obtained and the bone–implant contact (BIC), peri-implant osteoid volume and mineralized bone volume (MBV) were calculated after 1, 2 and 4 weeks. Using the same models mechanical push-in tests were also performed to evaluate the implant fixation strength. After 1 week the MBV and push-in strength were significantly lower in the 300 μg fluvastatin-treated group than in the other groups ($P < 0.01$). At 2 weeks, however, the BIC and MBV were both significantly higher in the 75 μg fluvastatin-treated group than in the non-fluvastatin-treated groups ($P < 0.01$). Similar tendencies were observed at week 4. Furthermore, the data showed a good correlation between the MBV and the push-in strength. These results demonstrate positive effects of locally applied fluvastatin on the bone around titanium implants and suggest that this improvement in osseointegration may be attributed to calcification of the peri-implant bone.

1. Introduction

A decline in tooth function is a serious problem for health and quality of life. Oral implants are now a well-accepted treatment in occlusal reconstruction [1,2]. It is well known that stability of implants is essential to achieve early osseointegration. Suitable control of the bone volume and bone quality around an implant is necessary for successful dental implant treatment. Titanium implants have been widely used clinically and many studies have been performed on bone formation surrounding implants using various growth factors [3–5]. However, it is still desirable to develop a strategy for improving osteogenesis around titanium implants.

Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors ("statins"), which inhibit a key enzyme in the mevalonate pathway, are widely used for the treatment of hyperlipidemia. Mundy et al. [6] first reported that statins in vitro induce the expression of bone morphogenetic protein-2 (BMP-2) mRNA in osteoblasts and stimulate bone formation in organ cultures of murine calvarial bone. Since then, several reports have shown that statins in vitro also promote osteoblast differentiation and mineralization [7,8] and stimulate expression of BMP-2 [9,10]. Several other animal studies have shown that statins promote the healing of bone fractures [11] and stimulate local bone formation when used with a carrier [12–16]. Furthermore, recent clinical studies have shown that statin use is associated with increased bone mineral density [17] and a reduced risk of hip fracture [18,19].

Our previous study showed that systemic application of simvastatin successfully activated osteogenesis around titanium implants in rat tibiae [20]. In the field of dentistry, however, implant therapies require focused effects specifically around the implants, thereby suggesting the importance of local application. In a previous study we found that topical application of fluvastatin increased bone formation and suppressed osteoclast activity at bone healing sites [21]. In consideration of these findings, we hypothesized that local application of statins would promote peri-implant bone formation, and recently demonstrated that topical application of statins successfully activated osteogenesis around titanium implants [22]. The purpose of the present study was to evaluate bone healing around titanium implants, especially bone quality, quantity and function, after local application of fluvastatin in a rat model.

* Corresponding author. Tel.: +81 92 642 6441; fax: +81 92 642 6380.
E-mail address: kabay@dent.kyushu-u.ac.jp (Y. Moriyama).

1742-7061/$ - see front matter © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2009.10.045

2. Materials and methods

2.1. Animals and assignment of control and test animals

Ten-week-old female Wistar rats weighing 200–250 g (Kyudo, Tosu, Japan) were used in this study. The rats were treated in accordance with our institute's guidelines for animal care (Kyushu University, Fukuoka, Japan). For this study the rats were housed under identical conditions and fed a commercially available standard rodent food containing 1.25% calcium, 1.06% phosphate and 2.0 IU g^{-1} vitamin D_3 (CE-2, CLEA Japan Inc., Tokyo, Japan). Water was available ad libitum.

As described below, the same concentrations of statin were applied to both the right and left tibiae of four rats for histological and histomorphometric analyses. In other words, we obtained eight similar samples in each group. Although it may be more appropriate to assign one tibia as a control bone and the other as an experimental bone, we were forced to create control animals (no statin application to both tibiae) and experimental animals (same concentration of statin applied to both tibiae) because the statin can move within the blood circulation. The assignment of control and test animals in the present study was based on a previous study [23].

2.2. Implants and statin

Pure titanium implants, 1 mm in diameter and 1.5 mm in length (average surface roughness 0.438 μm) (Kyocera, Kyoto, Japan), were sterilized with 95% ethanol and air dried before implantation. The required doses of fluvastatin (Toronto Research Chemicals Inc., North York, Canada) were obtained by dilution with phosphate buffer to 0.1, 0.5, 2.5 and 10 mg ml^{-1}. Propylene glycol alginate (PGA) (Wako Pure Chemical Industries, Osaka, Japan) was used as a carrier for local application (0.03 ml each) around the implants.

2.3. Implantation

Implantation was performed under general anesthesia using pentobarbital sodium, which was further supplemented as necessary. Each tibia was initially scrubbed with alcohol and exposed, before holes (1 mm in diameter) were made at 10 mm below the knee joint. After flushing the cavity with sterile saline, 0.03 ml of PGA gel alone or PGA gel containing each dose of fluvastatin was injected into the cavity. An implant was then inserted into each hole. The following groups were examined: implant only group; implant + PGA group; 3 μg FS group (implant + PGA containing 3 μg fluvastatin); 15 μg FS group (implant + PGA containing 15 μg fluvastatin); 75 μg FS group (implant + PGA containing 75 μg fluvastatin); 300 μg FS group (implant + PGA containing 300 μg fluvastatin). The wound was then sutured. Each group contained 12 animals and 24 tibiae. After 1, 2 and 4 weeks of treatment the animals were killed and subjected to perfusion fixation. The tibiae for histology were harvested without soft tissues and placed in fixation solution consisting of 0.1 M phosphate-buffered saline (pH 7.4) containing 5% glutaraldehyde and 4% paraformaldehyde. The specimens were dehydrated in progressively more concentrated ethyl alcohol solutions (50–100%) and then embedded in methylmethacrylate (Wako Pure Chemical Industries). Undecalcified ground sections at a thickness of approximately 70 μm parallel to the long axis of the implant (EXAKT Cutting & Grinding System; Exakt Apparatebau, Norderstedt, Germany) were fabricated as reported previously [20]. An outline of the experimental study is shown in Fig. 1.

2.4. Determination of peri-implant bone volume and bone–implant contact

The specimens were subjected to Villanueva–Goldner staining to distinguish between osteoid and mineralized bone. In this method mineralized bone is stained green while osteoid is stained red. Images of the stained sections were obtained by light microscopy and bone–implant contact (BIC) within 0.5 mm in the peri-implant osteoid volume (OV) and the mineralized bone volume (MBV) were calculated (Fig. 2). These histometric procedures were performed using NIH Image 1.62 (National Institutes of Health, Bethesda, MD).

2.5. Mechanical push-in test

Using the same models, 54 rats (108 tibiae) were killed with an overdose of ethyl ether at weeks 1, 2 and 4. Immediately after death the specimens and surrounding tissues were removed for mechanical test preparation. The mechanical push-in test to measure the strength of fixation of the implant was performed using a Universal Test Machine (Shimadzu, Kyoto, Japan) before the samples were dry [24]. Mechanical testing was accomplished at a rate of 0.25 mm min^{-1} and the force direction was parallel to the longitudinal axis of the implant (Fig. 3). The maximum value at the ultimate load was recorded during each push-in test.

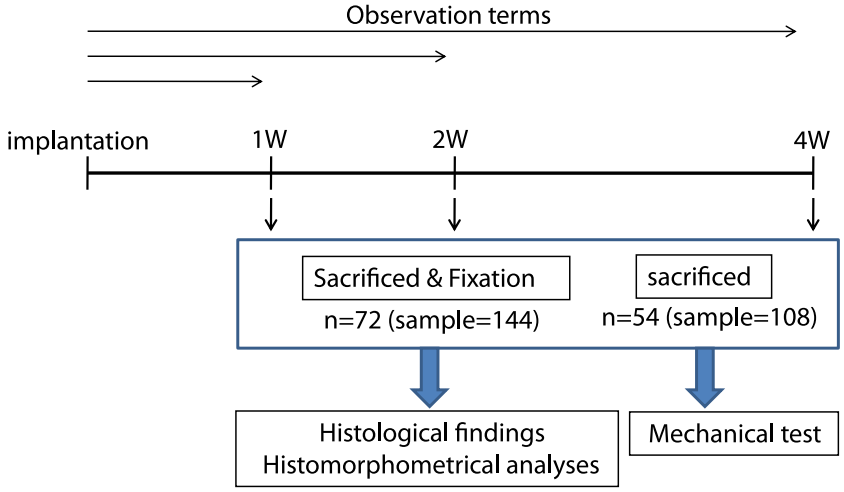

Fig. 1. Scheme of the time schedule in the present study.

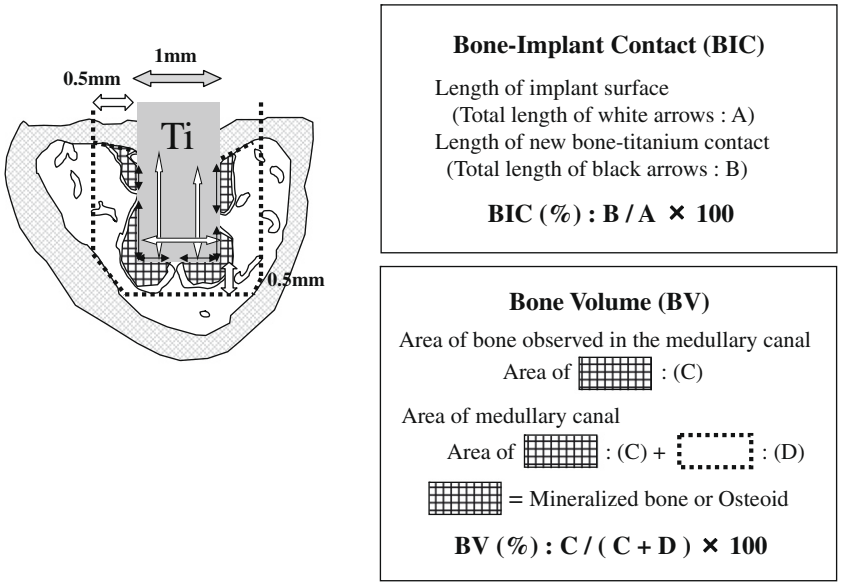

Fig. 2. Scheme for the calculation of bone–implant contact (BIC) and bone volume (BV). The percentage of new bone–titanium contact along the total length of the implant surface in the medullary canal is termed the BIC. The percentage of bone in the 0.5 mm surrounding the implant in the medullary canal is termed the BV. The BV was divided between the osteoid volume (OV) and the mineralized bone volume (MBV).

2.6. Statistical analyses

The morphometric and mechanical data were confirmed to be normally distributed by the Friedmann test and then analyzed by two-way analysis of variance for all groups. In addition, Pearson correlation coefficients between the morphometric and mechanical data were calculated. All analyses were performed using SPSS software (SPSS Japan, Tokyo, Japan).

2.7. The nature of the blinding

One researcher who was unaware of the groups to which the specimens belonged carried out all the histomorphometric measurements and push-in strength tests.

3. Results

3.1. Histological findings

At 1 week after implantation newly formed bone was observed around the titanium implants in all groups. The implant surfaces in the experimental groups were partially covered with a trabecular bone layer that was also connected to the peri-implant cortical bone (Fig. 4). In the 300 μg FS group, however, the implants were mostly covered with unmineralized osteoid, unlike the other experimental groups (Fig. 4f).

At week 2 abundant bone trabeculae with a mesh-like structure were seen in the medullary canal in the experimental groups (Fig. 5). In the 75 μg FS group in particular the bone trabeculae observed in the medullary canal were somewhat thicker than those

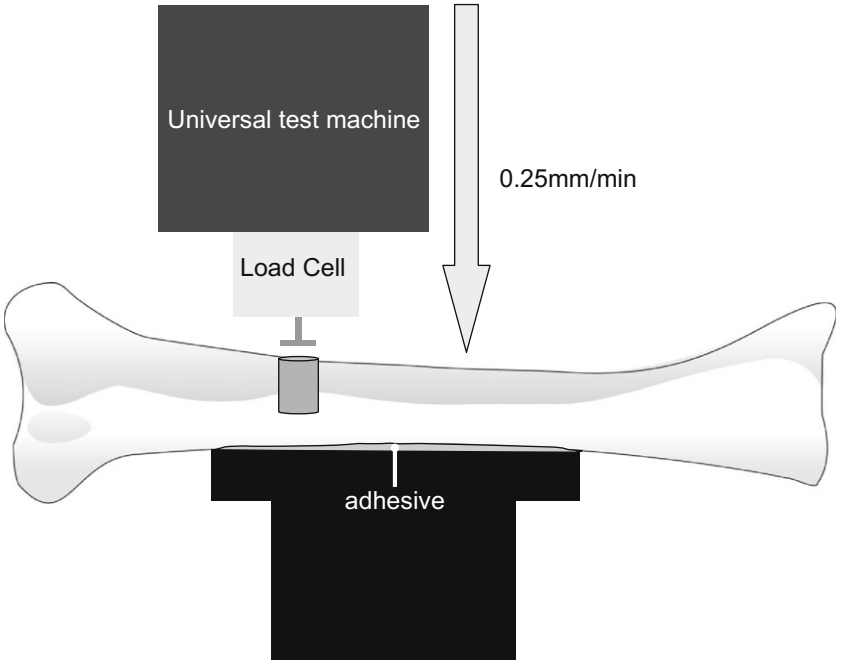

Fig. 3. Scheme of the mechanical push-in test.

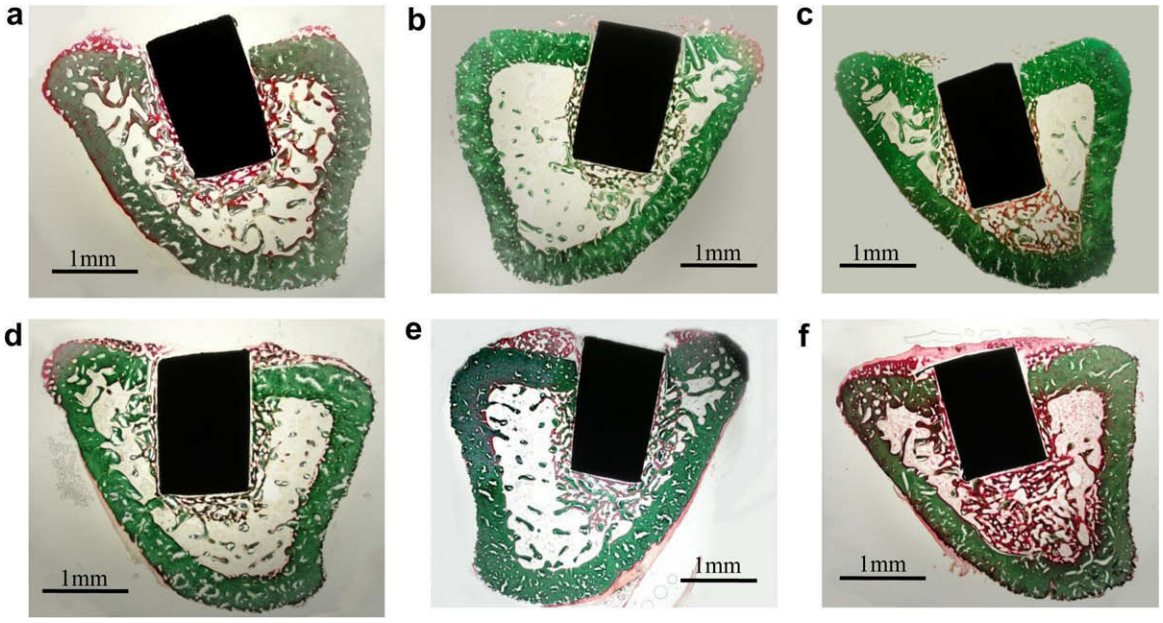

Fig. 4. Histological sections of tibiae at 1 week after implantation, stained by the Villanueva–Goldner method. In the bone matrix red staining represents osteoid bone and green staining represents mineralized bone. (a) Implant only. (b) Implant with PGA. (c) Implant with PGA containing 3 μg FS. (d) Implant with PGA containing 15 μg FS. (e) Implant with PGA containing 75 μg FS. (f) Implant with PGA containing 300 μg FS. There is new bone formation around the implants in all the groups; however, only the peri-implant new bone in the implant only group and 300 μg FS group is osteoid bone.

in the non-statin-treated groups. This newly formed trabecular bone layer comprised mineralized bone, since it stained green (Fig. 5e).

At week 4 newly formed bone that was also mineralized was observed around the implants in all groups (Fig. 6).

3.2. Histomorphometric findings

At week 1 there were no significant differences in the amounts of BIC among the groups (Fig. 7a). However, the OV was significantly higher in the 300 μg FS group than in the other groups ($P < 0.05$) (Fig. 8a). The MBV was significantly lower in the 300 μg FS group than in the 75 μg FS group ($P < 0.01$) (Fig. 9a).

At week 2 the BIC was significantly higher in both the 15 μg FS and 75 μg FS groups than in the implant only group ($P < 0.01$) (Fig. 7b). The peri-implant OV was significantly decreased in the 300 μg FS group compared with the implant only group ($P < 0.05$) (Fig. 8b). However, the MBV in the 75 μg FS group was significantly increased compared with the implant + PGA group ($P < 0.05$) (Fig. 9b).

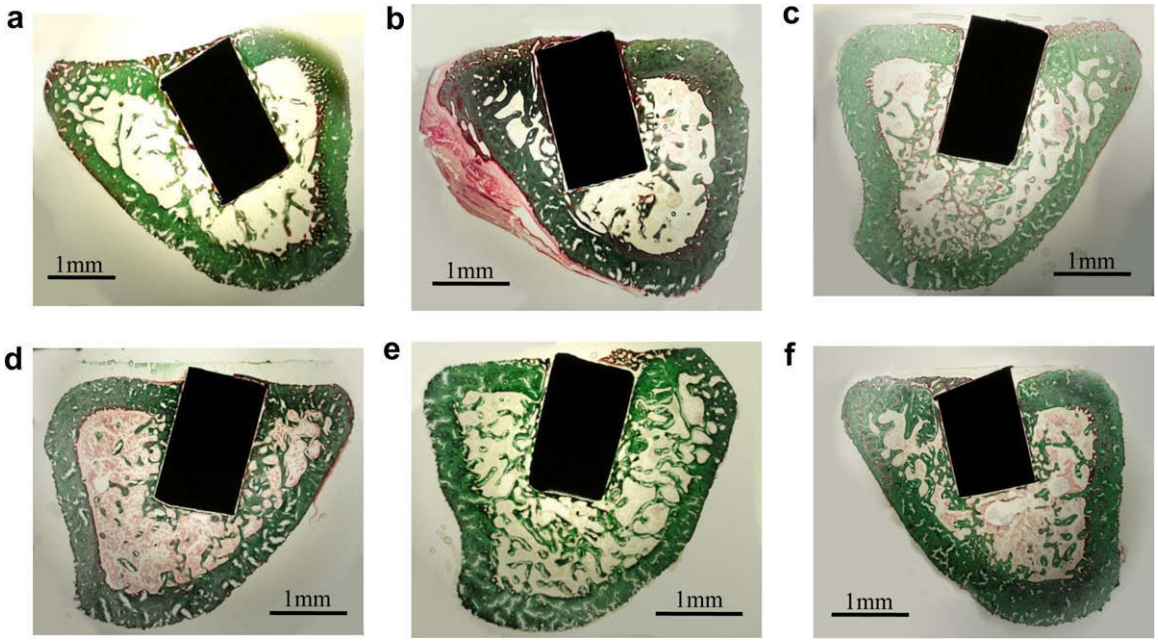

Fig. 5. Histological sections of tibiae at 2 weeks after implantation, stained by the Villanueva–Goldner method. In the bone matrix red staining represents osteoid bone and green staining represents mineralized bone. (a) Implant only. (b) Implant with PGA. (c) Implant with PGA containing 3 μg FS. (d) Implant with PGA containing 15 μg FS. (e) Implant with PGA containing 75 μg FS. (f) Implant with PGA containing 300 μg FS. Newly formed bone is observed around the titanium implants. In the fluvastatin treatment groups the medullary canal is filled with abundant bone trabeculae with a mesh-like structure.

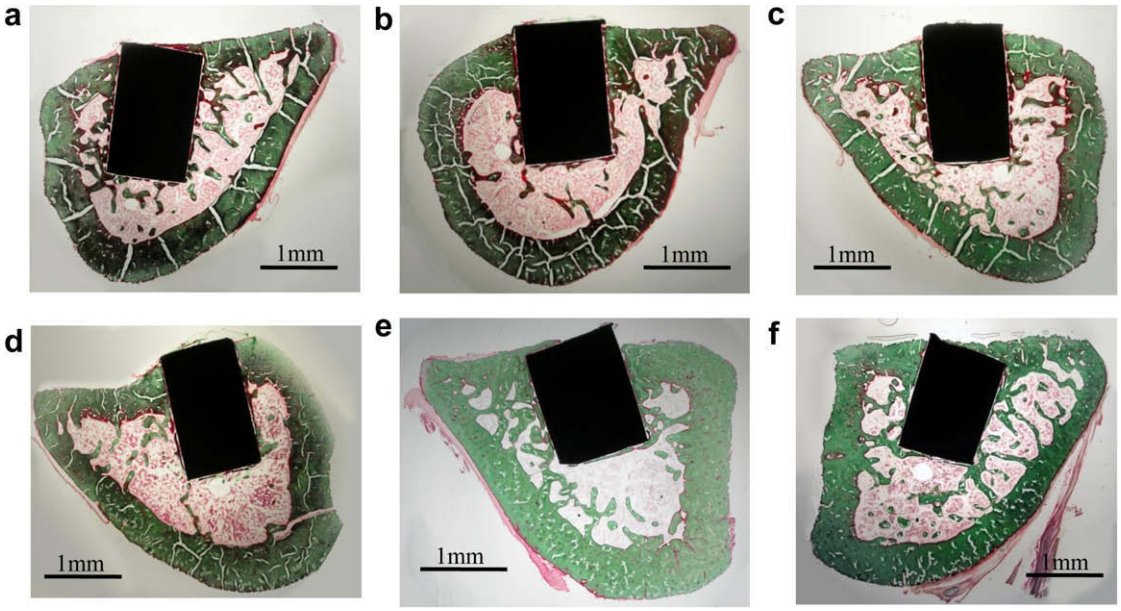

Fig. 6. Histological sections of tibiae at 4 weeks after implantation, stained by the Villanueva–Goldner method. In the bone matrix red staining represents osteoid bone and green staining represents mineralized bone. (a) Implant only. (b) Implant with PGA. (c) Implant with PGA containing 3 μg FS. (d) Implant with PGA containing 15 μg FS. (e) Implant with PGA containing 75 μg FS. (f) Implant with PGA containing 300 μg FS. There is new bone formation around the implant in all the groups; however, convergence of bone formation can be seen.

At week 4 there were no significant differences in the BIC (Fig. 7c), OV (Fig. 8c) and MBV (Fig. 9c) among the groups.

3.3. Implant fixation

The mechanical push-in results showed a significant increase in the 75 μg FS group compared with the implant only group ($P < 0.05$) at week 1 (Fig. 10a). At week 2, however, there was a significant increase in the 75 μg FS group compared with both the non-statin-treated groups ($P < 0.05$) (Fig. 10b). A non-significant trend of elevated strength was detected among the groups at week 4 (Fig. 10c).

Based on the above data, the MBV at weeks 1 and 2 and the BIC at week 2 were all positively correlated with the push-in strength (Pearson correlation coefficients of 0.477, 0.739 and 0.457, respectively). The OV at week 2 was negatively correlated with the push-in strength (Pearson correlation coefficient of −0.432) (Table 1).

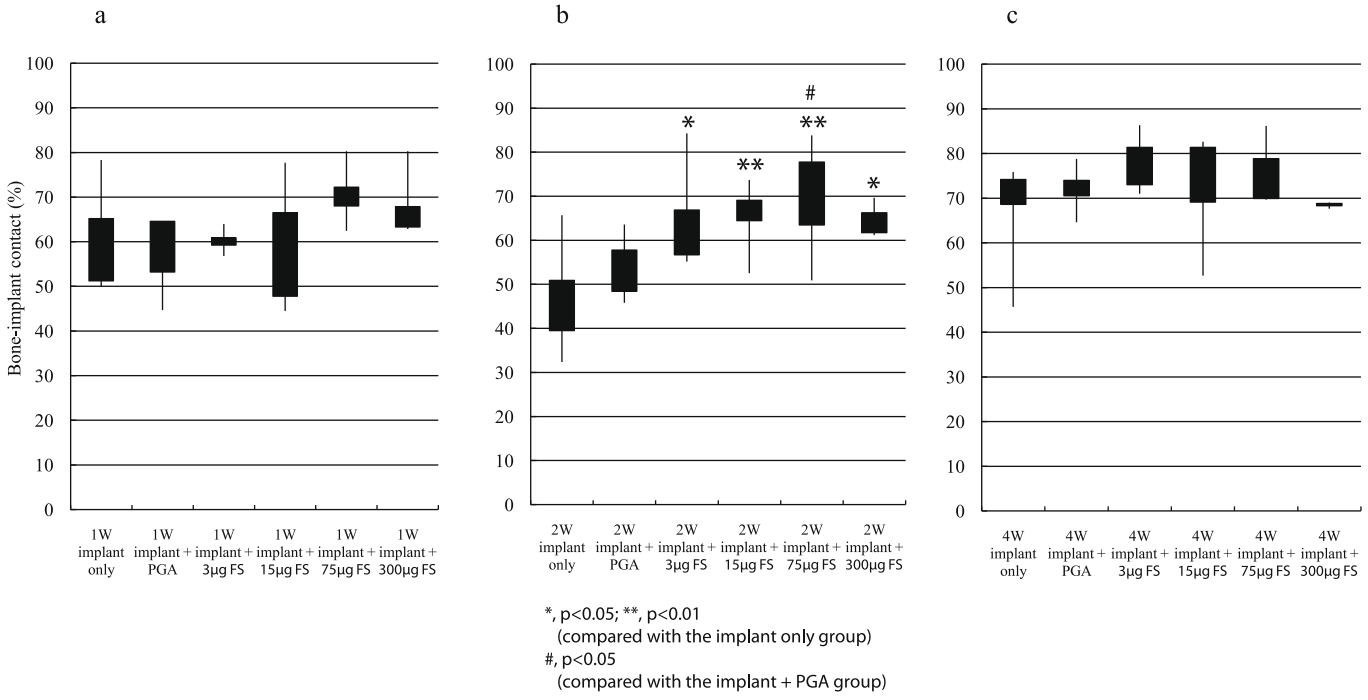

Fig. 7. Bone–implant contact (BIC). (a) BIC at week 1. There were no significant differences among the groups. (b) BIC at week 2. The BIC was significantly higher in the fluvastatin treatment groups than in the implant only group. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. the implant only group; $^{\#}P < 0.05$ vs. the implant + PGA group. (c) BIC at week 4. There were no significant differences among the groups.

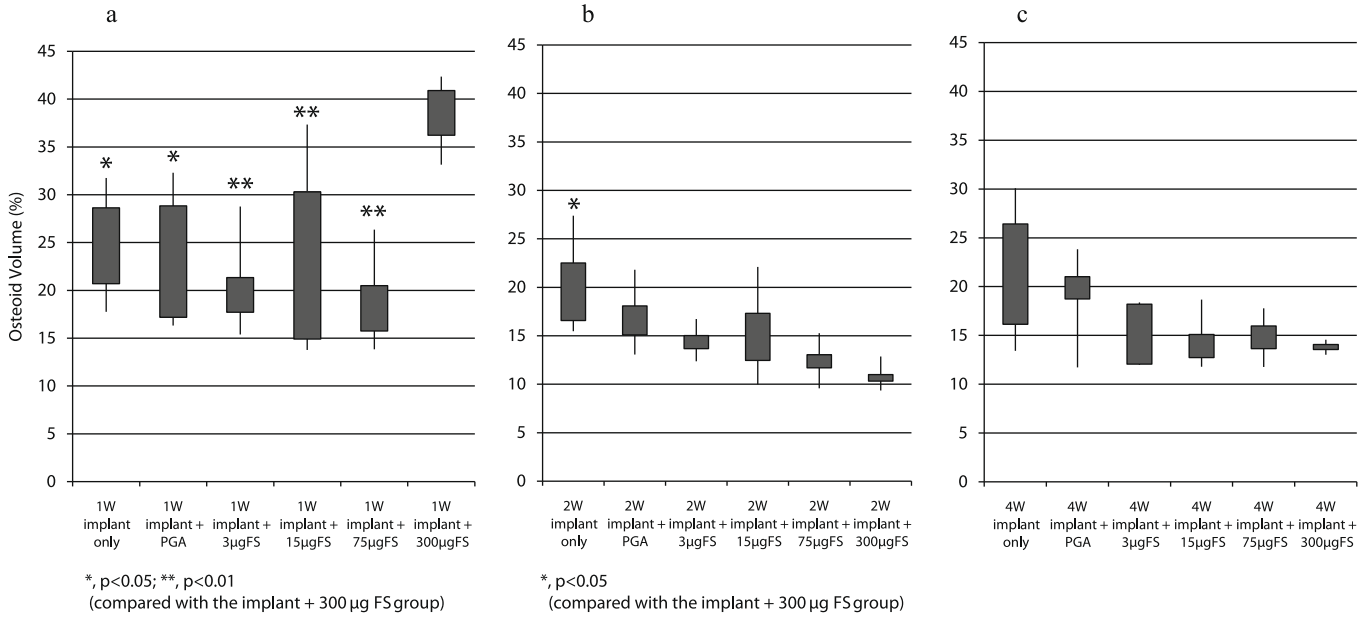

Fig. 8. Osteoid volume (OV). (a) OV at week 1. There was a significant increase in the 300 μg FS group compared with the other groups. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. the 300 μg FS group. (b) OV at week 2. There is a significantly higher increase in the control group than in the 300 μg FS group. $^{*}P < 0.05$ vs. the 300 μg FS group. (c) OV at week 4. There were no significant differences among the groups.

4. Discussion

Stimulation of local bone formation is an important factor in the repair of bony defects in dentistry. In recent years several substances have been used to progress peri-implant bone formation, including bone morphogenetic proteins [3,25], growth factors (e.g. transforming growth factor [5] and fibroblast growth factor [26,27]), drugs (e.g. bisphosphonate [28]) and hormones (e.g. growth hormone [4]). However, these substances have not yet been used clinically. Clinical application of gene therapies and growth factors appear to be unsuitable at present.

We have been focusing on statins. Although statins have been reported to have certain side-effects, such as rhabdomyolysis, the incidences of these side-effects are very low and statins have been widely used on hyperlipidemic patients. Statins have been shown to exert pleiotropic effects, such as anti-inflammatory, antioxidant, antithrombotic, immunomodulatory and angiogenic effects [29]. In particular, statins were previously reported to promote bone formation in vitro and in vivo by augmenting the expression of BMP-2 [6,9–11,30]. Several studies have indicated that the use of statins in humans may be associated with increased bone mineral density [17] and a reduced fracture risk [18,19]. In contrast, nega-

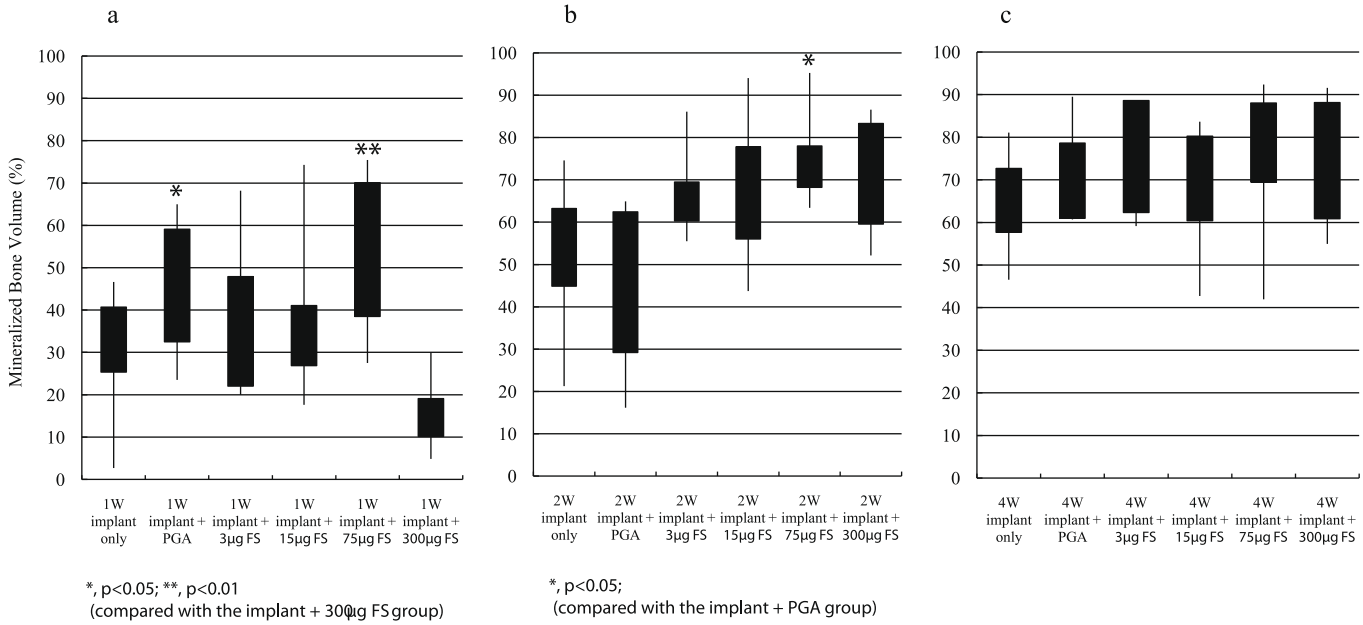

Fig. 9. Mineralized bone volume (MBV). (a) MBV at week 1. There was a significant decrease in the 300 μg FS group compared with the 75 μg FS group and implant + PGA group. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. the 300 μg FS group. (b) MBV at week 2. There was a significant increase in the 75 μg FS group compared with the implant + PGA group. $^{*}P < 0.05$ vs. the implant + PGA group. (c) MBV at week 4. There were no significant differences among the groups.

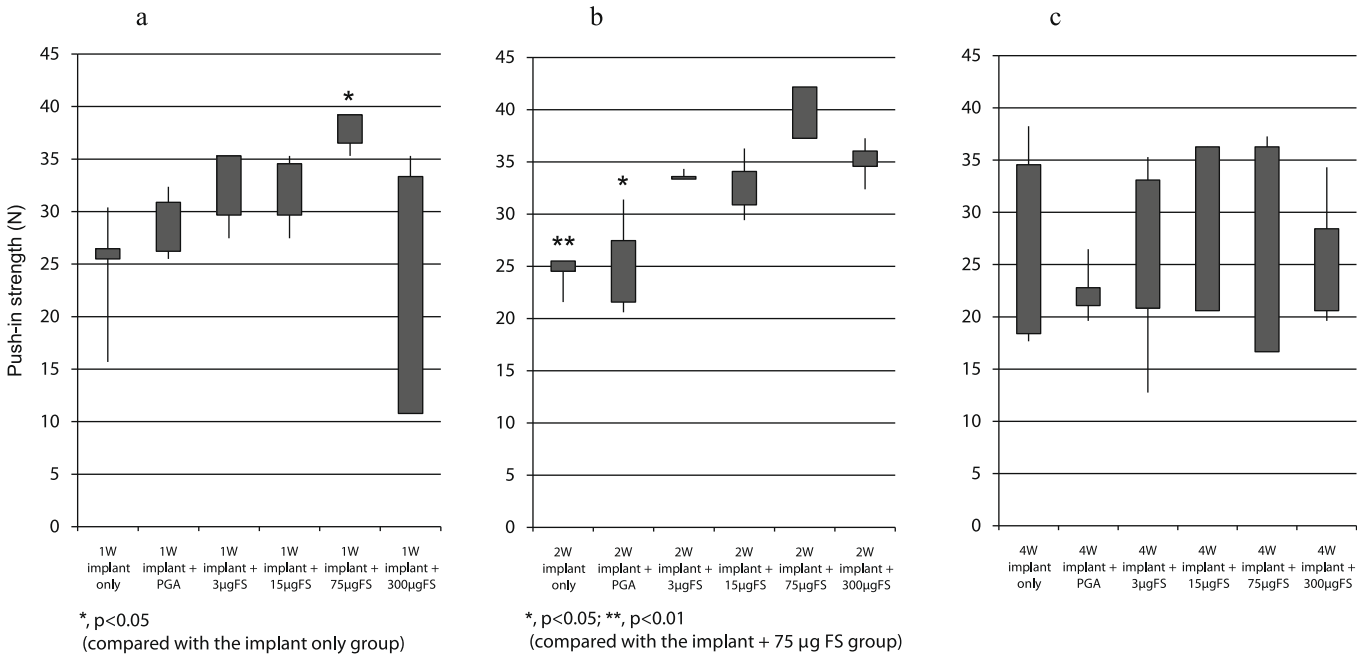

Fig. 10. Mechanical push-in strength. (a) Push-in strength at week 1. There was a significant decrease in the implant only group compared with the 75 μg FS group. $^{*}P < 0.05$ vs. the implant only group. (b) Push-in strength at week 2. There was a significant increase in the 75 μg FS group compared with the non-statin-treated groups. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. the 75 μg FS group. (c) Push-in strength at week 4. There were no significant differences among the groups.

Table 1
Pearson correlation coefficients between the push-in strength and the histomorphometric parameters.

		Mineralized bone volume	Osteoid volume	Bone implant contact
Push-in strength	1W	0.477	0.221	0.146
	2W	0.739	−0.432	0.457
	4W	0.216	0.216	−0.306

Pearson correlation coefficients, $P < 0.05$.
Significant correlations were observed between the push-out strength and the MBV at weeks 1 and 2 and the BIC and the OV at week 2.

tive findings have also been reported in animal studies [31,32] and clinical studies [33,34]. Therefore, it is unknown whether the use of statins is associated with anabolic effects on bone in vivo. The reason for this uncertainty may be that most experimental designs administer statins orally and they are subsequently metabolized in the liver. Consequently, only small amounts of statins pass through the liver to the bones [35].

There is a possibility that statins promote osteogenesis around implants and we have previously demonstrated that systemic administration of simvastatin increases the BIC and BV around titanium implants [20]. However, when using systemic simvastatin treatment in our rodent models the doses were about 10-fold higher than the dose presently recommended for lowering cholesterol. Although there were no confirmed side-effects, such as inflammatory responses, implant therapies require focused effects in specific regions, thereby suggesting the importance of topical application. To accomplish this, several studies have demonstrated bone formation after local application of statins [12–16] and we have shown that topical application of simvastatin or fluvastatin increased bone formation and inhibited osteoclast activation at bone healing sites [21]. Therefore, local statin delivery may represent an interesting approach to bring the drug into immediate contact with the peri-implant bone. In the present study we evaluated the possibility of using titanium implants as a statin carrier in a rat model.

As mentioned above, several studies have demonstrated the osteogenic effects of locally applied statins. Several kinds of carriers were employed in these studies, such as methylcellulose gels [12,16], collagen sponges [13], calcium sulfate [15] and biodegradable polymer nanobeads of poly(lactic-co-glycolide acid) [36]. In our previous study we used α-TCP to apply fluvastatin to bone [37]. In this study we used four different doses of fluvastatin to estimate the optimal dosage and administered the drug locally with PGA at implant sites in rat tibiae.

PGA is a vehicle that is clinically used as a carrier for enamel matrix derivative (EMD). Gestrelius et al. [38] demonstrated that EMD in PGA solution has a half-life of 2–3 days and that remnants of EMD can be present for 2 weeks. In the present study the rate of degradation of PGA and sustained release of fluvastatin were not examined. These aspects need to be elucidated in the future. However, our present results demonstrate that there were large amounts of mineralized bone around the titanium implants at week 2. These results agree with the findings of Gestrelius et al. [38].

There are some details that differ between our previous study [22] and the present one. In addition to our pilot study [22], we examined the optimal concentration for local application around an implant and administered an excess dose of fluvastatin (300 μg) in the present study. Furthermore, to elucidate the bone quality of the new bone around the implants we employed the Villanueva–Goldner staining method, by which we could distinguish between mineralized bone and osteoid, in the present study. In the histomorphometric findings at week 2 local application of fluvastatin enhanced mineralized bone formation around the titanium implants as well as the BIC. Our previous study [21] showed that local application of fluvastatin at an early stage of bone healing provoked down-regulation of cathepsin K, an osteolytic enzyme that is specifically produced by osteoclasts. Accordingly, our data at week 2 suggest that the functions of osteoclasts were inhibited by fluvastatin and the new trabeculae in the medullary canal were retained. Furthermore, although no statistical analyses were carried out, these trabeculae appeared to be thicker and, as a

result, the MBV became elevated. At week 1, however, an excess dose (300 μg) of fluvastatin produced a significant delay in calcification of the peri-implant bone. Our previous study [21] also demonstrated that both BMP-2 and alkaline phosphatase were significantly increased, whereas osteocalcin (OCN) showed no difference. According to a previous report, Runx2, an important member of the BMP-2 signaling pathway, is essential for osteoblastic differentiation [39]. However, for the final differentiation of osteoblasts, which is characterized by OCN expression, the coordination of Runx2 suppression and Osterix enhancement is necessary [39]. Although we did not evaluate their expression, statins are suggested to stimulate Runx2 expression, and this may be a possible cause of OCN suppression. In our results we demonstrated that the peri-implant bone stained red in the highest dose statin group, indicating that the bone was not mineralized. We hypothesize that the peri-implant bone in the highest dose group was produced by immature osteoblasts. Taking the results for mechanical strength into consideration, it is suggested that the improvement in osseointegration may cause calcification of the peri-implant bone and a small proportion of the OV. Furthermore, we examined the correlations between the histomorphometric data (peri-implant MBV, OV and BIC) and push-in strength. The data provided by our mechanical push-in test procedure may include not only shear forces between the bone and the side surface of the implants but also the breaking force of the bone at the bottom of the implants. This may be the reason why the correlations between the data from the histological analyses and the mechanical testing were not necessarily very strong. Nevertheless, the results showed good correlations of the push-in strength with both the MBV and BIC at 2 weeks. It is likely that the use of fluvastatin did have an effect on the mechanical properties of the implants. On the other hand, in terms of the mechanical strength at week 4, there were no significant differences among the groups. In the traditional concept there is a strong association between the BIC and implant fixation. However, in this study the amount of BIC showed no difference among the groups, but we found a link between the MBV and implant fixation. Application of fluvastatin improved initial fixation of the implants by promoting the early stage of mineralized bone formation around the implants.

At present dental implant treatments are frequently performed, highly predictable procedures. In the early stages a lack of primary stability seems to be one of the important causes of implant failure. It would therefore seem to be favorable to improve primary healing around the implant [40]. This study has shown that both the early stages of mineralized bone formation around titanium implants and implant fixation were improved by application of fluvastatin.

There were some limitations in the present study. First, we employed a rat tibia model. Thus occlusal force and other stresses did not act around the implants. In addition, the bone structure of the tibia is different from that of the jaw. From the point of view of the pharmacokinetics, differences in the reaction to statins between humans and rodents remain to be elucidated. From the point of view of the push-in tests, the data may include both the shear force between the bone and the side surface of the implants and the breaking force of the bone at the bottom of the implants, as mentioned above. Therefore, the adequacy of this method should be further investigated. Accordingly, the effects of statins on the bone around implants inserted into the jaw bone require further study using improved mechano-biological procedures.

5. Conclusions

The present study has clearly demonstrated that intramedullary application of fluvastatin can enhance osteogenesis around titanium implants at an early stage (2 weeks) in this rat model. Therefore, statins should be further examined for use in implant treatments.

Acknowledgement

This work was supported in part by KAKENHI (Nos. 14370638 and 18390518) from the Japan Society for the Promotion of Science.

Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figure 1, are difficult to interpret in black and white. The full color images can be found in the on-line version, at doi: 10.1016/j.actbio.2009.10.045.

References

[1] Albrektsson T, Dahl E, Enbom L, Engevall S, Engquist B, Eriksson AR, et al. Osseointegrated oral implants. A Swedish multicenter study of 8139 consecutively inserted Nobelpharma implants. J Periodontol 1988;59:287–96.
[2] Adell R, Eriksson B, Lekholm U, Brånemark PI, Jemt T. A long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants 1990;10:347–59.
[3] Scmidmaier G, Wildemann B, Cromme F, Kandziora F, Haas NP, Raschke M. Bone morphogenetic protein-2 coating of titanium implants increases biomechanical strength and accelerates bone remodeling in fracture treatment: a biomechanical and histological study in rats. Bone 2002;30:816–22.
[4] Tresguerres IF, Blanco L, Clemente C, Tresguerres JAF. Effects of local administration of growth hormone in peri-implant bone: an experimental study with implants in rabbit tibiae. Int J Oral Maxillofac Implants 2003;18:807–11.
[5] De Ranieri A, Virdi AS, Kuroda S, Shott S, Leven RM, Hallab NJ, et al. Local application of rhTGF-β2 enhances peri-implant bone volume and bone–implant contact in a rat model. Bone 2005;37:55–62.
[6] Mundy GR, Garrett R, Harris S, Chan J, Chen D, Rossini G, et al. Stimulation of bone formation in vitro and in rodents by statins. Science 1999;286:1946–9.
[7] Maeda T, Matsunuma A, Kawane T, Horiuchi N. Simvastatin promotes osteoblast differentiation and mineralization in MT3T3-E1 cells. Biochem Biophys Res Commun 2001;280:874–7.
[8] Song C, Guo Z, Ma Q, Chen Z, Liu Z, Jia H, et al. Simvastatin induces osteoblastic differentiation and inhibits adipocytic differentiation in mouse bone marrow stromal cells. Biochem Biophys Res Commun 2003;308:458–62.
[9] Sugiyama M, Komada T, Konishi K, Abe K, Asami S, Oikawa S. Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun 2000;271:688–92.
[10] Ohnaka K, Shimoda S, Nawata H, Shimokawa H, Kaibuchi K, Iwamoto Y, et al. Pitavastatin enhanced BMP-2 and osteocalcin expression by inhibition of Rho-associated kinase in human osteoblasts. Biochem Biophys Res Commun 2001;287:337–42.
[11] Skoglund B, Forslund C, Aspenberg P. Simvastatin improves fracture healing in mice. J Bone Miner Res 2002;17:2004–8.
[12] Thylin MR, McConnel JC, Schmid MJ, Reckling RR, Ojha J, Bhattacharyya I, et al. Effects of simvastatin gels on murine calvarial bone. J Periodontol 2002;73:1141–8.
[13] Wong RWK, Rabie ABM. Statin collagen grafts used to repair defects in the parietal bone of rabbits. J Oral Maxillofac Surg 2003;41:244–8.
[14] Galus R, Wlodarski P, Wlodarski KH. Fluvastatin increases heterotopically induced ossicles in mice. Clin Exp Pharmacol Physiol 2006;33:388–90.
[15] Nyan M, Sato D, Oda M, Machida T, Kobayashi H, Nakamura T, et al. Bone formation with the combination of simvastatin and calcium sulfate in critical-sized rat calvarial defect. J Pharmacol Sci 2007;104:384–6.
[16] Lee Y, Schmid MJ, Marx DB, Beatty MW, Cullen DM, Collins ME, et al. The effect of local simvastatin delivery strategies on mandibular bone formation in vivo. Biomaterials 2008;29:1940–9.
[17] Edward CJ, Hart DJ, Sector TD. Oral statins and increased bone-mineral density in postmenopausal women. Lancet 2000;355:2218–9.
[18] Chan KA, Andrade SE, Boles M, Buist DS, Chase GA, Donahue JG, et al. Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fractures among older women. Lancet 2000;355:2185–8.
[19] Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H. HMG-CoA reductase inhibitors and the risk of fractures. J Am Med Assoc 2000;283:3205–10.
[20] Ayukawa Y, Okamura A, Koyano K. Simvastatin promotes osteogenesis around titanium implants. A histological and histometrical study in rats. Clin Oral Implants Res 2004;15:346–50.
[21] Ayukawa Y, Yasukawa E, Moriyama Y, Ogino Y, Wada H, Atsuta I, et al. Local application of statin promotes bone repair through the suppression of osteoclasts and the enhancement of osteoblasts at bone-healing sites in rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:336–42.

[22] Moriyama Y, Ayukawa Y, Ogino Y, Atsuta I, Koyano K. Topical application of statin affects bone healing around implants. Clin Oral Implants Res 2008;19:600–5.
[23] Kajiwara H, Yamaza T, Yoshinari M, Goto T, Iyama S, Atsuta I, et al. The bisphosphonate pamidronate on the surface of titanium stimulates bone formation around tibial implants in rats. Biomaterials 2005;26:581–7.
[24] Lopes MA, Santos JD, Monteiro FJ, Ohtsuki C, Osaka A, Kaneko S, et al. Push-out testing and histological evaluation of glass reinforced hydroxyapatite composites implanted in the tibia of rabbits. J Biomed Mater Res 2001;54:463–9.
[25] Sykaras N, Iacopino AM, Triplett RG, Marker VA. Effect of recombinant human bone morphogenetic protein-2 on the osseointegration of dental implants: a biomechanics study. Clin Oral Invest 2004;8:196–205.
[26] Stenport VF, Johansson CB, Sawase T, Yamasaki Y, Oida S. FGF-4 and titanium implants: a pilot study in rabbit bone. Clin Oral Implants Res 2003;14:363–8.
[27] Akagawa Y, Kubo T, Koretake K, Hayashi K, Doi K, Matsuura A, et al. Initial bone regeneration around fenestrated implants in Beagle dogs using basic fibroblast growth factor–gelatin hydrogel complex with varying biodegradation rates. J Prosthodont Res 2009;53:41–7.
[28] Meraw SJ, Reeve CM, Wollan PC. Use of alendronate in peri-implant defect regeneration. J Periodontol 1999;70:151–8.
[29] Horiuchi N, Maeda T. Statin and bone metabolism. Oral Dis 2006;12:85–101.
[30] Garrett IR, Gutierrez G, Mundy GR. Statins and bone formation. Curr Pharm Des 2001;7:715–36.
[31] von Stechow D, Fish S, Yahalom D, Bab I, Chorev M, Muller R, et al. Does simvastatin stimulate bone formation in vivo? BMC Musculoskelet Disord 2003;4:8.
[32] Maritz FJ, Conradie MM, Hulley PA, Gopal R, Hough S. Effects of statins on bone mineral density and bone histomorphometry in rodents. Arterioscler Thromb Vasc Biol 2001;21:1636–41.
[33] Van Staa TP, Wegman S, Leufkens B, Cooper C. Use of statins and risk of fractures. J Am Med Assoc 2001;285:1850–5.
[34] Reid IR, Tonkin A, Cannon CP. Comparison of the effects of pravastatin and atorvastatin on fracture incidence in the PROVE IT-TIMI 22 trial—secondary analysis of a randomized controlled trial. Bone 2005;37:190–1.
[35] Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F. New insights into the pharmacodynamic and pharmacokinetic proprieties of statins. Pharmacol Ther 1999;84:413–28.
[36] Garrett IR, Gutierrez GE, Rossini G, Nyman J, McCluskey B, Flores A, et al. Locally delivered lovastatin nanoparticles enhance fracture healing in rats. J Orthop Res 2007;25:1351–7.
[37] Jinno Y, Ayukawa Y, Ogino Y, Atsuta I, Tsukiyama Y, Koyano K. Vertical bone augmentation with fluvastatin in an injectable delivery system: a rat study. Clin Oral Implants Res 2009;20:756–60.
[38] Gestrelius S, Andersson C, Johansson AC, Persson E, Brodin A, Rydhag L, et al. Formulation of enamel matrix derivative for surface coating. J Clin Periodontol 1997;24:678–84.
[39] Komori T. Runx2, a multifunctional transcription factor in skeletal development. J Cell Biochem 2002;87:1–8.
[40] Lioubavina-Hack N, Lang NP, Karring T. Significance of primary stability for osseointegration of dental implants. Clin Oral Implants Res 2006;17:244–50.