The effects of a nonsteroidal anti-inflammatory drug on the degree of titanium implant osseointegration

Vesile Elif Toy^a, Serkan Dundar^{b,*}, Alihan Bozoglan^b

^a Inonu University, Faculty of Dentistry, Department of Periodontology, Malatya, Turkey

^b Firat University, Faculty of Dentistry, Department of Periodontology, Elazig, Turkey

ARTICLE INFO

Keywords:

Nonsteroidal anti-inflammatory drug
Diclofenac sodium
Osseointegration
Bone implant connection

ABSTRACT

Objective: The aim of this study was to histologically examine the effects of a nonsteroidal anti-inflammatory drug, namely diclofenac sodium (DCS), on the extent of the bone–implant contact (BIC) of titanium implants after four weeks of osseointegration period in a rodent model.

Material & methods: Fourteen female Sprague-Dawley rats were divided into two groups: the control (n = 7) and experimental (DCS) groups. Fourteen machine-surfaced titanium implants were placed in the right tibial bones of the rats. The DCS (2 mg/kg) was administered by means of oral gavage to the experimental group for 14 days after four weeks of osseointegration. No medication was administered to the control group throughout the six-week study period. At the end of the study, the rodents were sacrificed and block sections were obtained for histologic evaluation.

Results: The mean BIC ratios for the control and DCS groups were $64.15 \pm 6.31\%$ and $61.10 \pm 6.08\%$, respectively. No statistically significant difference in terms of the BIC ratios was found between the two groups.

Conclusion: The results of this study demonstrate that DCS did not impair the BIC of the implants after four weeks of osseointegration.

1. Introduction

The use of dental implant-supported prostheses is a scientifically sanctioned and thus common treatment for partial or total edentulism.¹ Dental implants have a good rate of success (ranging from 90 to 95%) when used in patients without risk factors.² However, implant failures do occur in certain circumstances. According to the American Academy of Periodontology, osseointegration is defined as a direct structural and functional connection, in addition to the absence of fibrous tissue, between living bone tissue and the surface of a load-bearing implant.³ Optimal bone healing during the early phases of osseointegration is required to ensure the success of a dental implant. The patient's systemic health, the surgical technique applied, as well as the quality and quantity of the bone all influence peri-implant bone healing. Certain medical conditions are known to affect both the quality and the quantity of existing bone.⁴

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used by patients worldwide to reduce the inflammatory burden and to control chronic pain. In fact, it has been estimated that 6% of the global population regularly use NSAIDs.⁵ Furthermore, NSAIDs are extensively prescribed to manage the postoperative pain and discomfort experienced after

dental surgery. It has been reported that NSAIDs exert an influence on bone healing through inhibiting the release of prostaglandin (PG), which modulates bone metabolism through the suppression of the cyclooxygenase (COX) pathway.⁶ The reduction in the secretion of PG leads to the inhibition of osteogenesis, as PG is a complex regulator of bone remodeling, which induces bone formation and resorption.⁷ In addition, NSAIDs have been reported to suppress the proliferation of osteoblast cells through inhibiting DNA synthesis and limiting the release of bone morphogenetic proteins (BMPs), thereby exerting an osteoinductive effect.⁸

The arachidonic acid that is released from the membrane phospholipids is converted into PG by the two COX subtypes, namely COX-1 and COX-2. COX-1 is a constitutive enzyme that plays a regulatory role in many physiological events, while COX-2 is expressed by a series of stimuli, including inflammation, mechanical stress, and injury. The release of COX-2 is induced by proinflammatory mediators, and it is the main enzyme that regulates the production of PG.^{9,10} The mechanism by which the COX inhibitors influence bone healing has thus far been only poorly elucidated, although it is known that both COX-1 and COX-2 are involved in the early stages of osteogenesis, while COX-2 is also known to be an important regulator of the maturation of osteoblasts

* Corresponding author. Firat University, Faculty of Dentistry, Department of Periodontology, 23119, Campus, Elazig, Turkey.

E-mail addresses: sdundar@firat.edu.tr, dtserkandundar@gmail.com (S. Dundar).

<https://doi.org/10.1016/j.jobcr.2020.06.006>

Received 10 February 2020; Received in revised form 27 May 2020; Accepted 18 June 2020

Available online 03 July 2020

2212-4268/ © 2020 Craniofacial Research Foundation. Published by Elsevier B.V. All rights reserved.

during later stages.^{8,11} The COX inhibitors may affect the prognosis of titanium implants in a similar way to how they affect bone metabolism, especially when they are used on a daily basis for a long period of time. Diclofenac sodium (DCS) is a non-specific COX inhibitor that inhibits both COX-1 and COX-2.¹² Although the COX inhibitors have been found to impair bone healing in various preclinical studies, the impact of NSAIDs on the osseointegration of titanium implants remains unclear.^{13–17} Therefore, the aim of the present study was to evaluate the effects of an NSAID, namely DCS, which is commonly used as an analgesic, on the extent of the bone–implant contact (BIC) after four weeks of osseointegration.

2. Material & methods

2.1. Animals and experimental design

The experimental and surgical protocols involved in the current study were performed at Firat University's Experimental Research Center, Elazığ, Turkey. Ethical consent to conduct the study was obtained from Firat University's committee on animal research and ethics (2017,99). The animals used in the study were obtained from Firat University's Experimental Research Center. This research fully complied with the requirements of the Declaration of Helsinki regarding animal experimentation.

Fourteen female Sprague-Dawley rats were used in the present study. The rats weighed between 280 g and 320 g, and they were aged 0.5–1.0 years. The rats were housed in temperature-controlled cages and subjected to a 12-h light/12-h dark cycle. They had free access to both food and water.

The rats were randomly divided into two groups, namely the control and experimental (DCS) groups. Titanium implants were surgically placed in the right tibial bones of the rats in both groups. The DCS (2 mg/kg) was administered to the experimental group by means of oral gavage for 14 days (two weeks) after four weeks of osseointegration. The oral gavage was preferred according to the approach detailed in Beck et al.'s study.¹⁵ No medication was administered to the control group throughout the six-week study period.

2.2. Surgical applications

The surgical procedures, which were all performed by the same researcher, were carried out under general anesthesia in sterile conditions. The general anesthesia was administered via an intramuscular injection of 40 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine. The skin was shaved and washed with povidone iodine prior to the surgery. A linear incision (1.5–2.0 cm in length) was made to the skin of the tibial crest. A periosteal elevator was then used to access the metaphyseal part of the tibial bone. The implant sockets were created using appropriate drills (i.e., a 1.8-mm drill bit initially, followed by a 2.3-mm drill bit) under sterile physiological serum perfusion. Following the creation of the bone sockets, 14 machine-surfaced titanium implants (2.5 mm in diameter and 4.5 mm in length) were placed in the corticocancellous part of the right tibial bones of the rodents. This was followed by the repositioning of the subcutaneous tissue and skin back into their original positions. Suturing was then performed using 4-0 polyglactin absorbable material. After the surgical procedures, all the rodents received an analgesic and antibiotics, which were administered intramuscularly for three days.

2.3. Histological procedures

All the rats were sacrificed six weeks after the surgical procedures. The implants were removed, along with the surrounding bone tissue. The specimens were then fixed in a 10% formaldehyde solution for seven days. After that, they were embedded in 2-hydroxyethylmetacrylate resin to enable the cutting of the undecalcified bone

and titanium using an EXAKT® microtome (EXAKT Advanced Technologies GmbH, Norderstedt, Germany). The implants with surrounding bone tissue were ground using an EXAKT® grinder (EXAKT Advanced Technologies GmbH) to allow for the histologic analysis. Further, sections (50 µm thick) were obtained for the light microscope analysis, which were stained using a 0.01% toluidine blue solution. All the histological procedures were performed in the research laboratory of the Faculty of Dentistry at the University of Erciyes, Kayseri, Turkey. The histologic analysis was performed using a light microscope (Nicon, Japan) obtained from the Department of Medical Microbiology, Faculty of Medicine, Firat University, Elazığ, Turkey. Finally, the BIC ratio was determined for each section as a proportion of the total implant surface length in direct contact with the bone.¹

2.4. Statistical analysis

SPSS® software, 22 (IBM, New York, USA) was used for all the statistical analyses in the present study. The data were analyzed, and the mean $\pm$ standard deviation was calculated. Student's *t*-test was used in the data analysis. Further, a *p*-value of < 0.050 was considered to be statistically significant.

3. Results

No fatal and nonfatal complications (such as wound formation and wound infection) were encountered during the experimental period generally. Neither undesired situation was encountered in the preparation of histological analysis of the specimens. When the samples were examined, it was seen that all implants were in the corticocancellous bone similar to the maxillary and mandibular bone tissues. Cortical and trabecular bone were evaluated together while performing BIC analyzes. Cortical bone was detected in the neck of the implants and trabecular bone in the surrounding part. In addition, bone formations were detected in the implant threads in accordance with the periimplant bone response. When the cortical bone was examined, it was determined that it was painted lighter blue, while the trabecular bone, in which maturation was not fully realized, was painted in a darker blue color. The histological BIC data concerning the control and DCS groups, which were obtained via the histologic analysis, are presented in Table 1. The mean BIC ratios for the control and DCS groups were $64.15 \pm 6.31\%$ and $61.10 \pm 6.08\%$, respectively. There was no statistically significant difference found between the two groups in terms of the BIC ratios ($P > 0.050$) (Fig. 1 a, b).

4. Discussion

The process of osseointegration around dental implants is similar to the process of bone healing, meaning that bone metabolism is crucial to the success of osseointegration. Therefore, it can be assumed that drugs capable of influencing bone metabolism might negatively affect osseointegration and thus the success rate of implants. Although impaired bone healing and bone metabolism are negatively associated with NSAID use, little is currently known about the impact of NSAIDs on the osseointegration of titanium implants.

NSAIDs are widely used to manage chronic diseases as well as to control postoperative pain and discomfort. Therefore, with a view to

Table 1
Bone implant connection ratios (%) of the groups. Statistically significant differences was not detected between the groups.

Groups	BIC (%) $\pm$ Standard Deviation	P ^a
DCS (n = 7)	61.10 $\pm$ 6.08	0.376 ($P > 0.05$)
Controls (n = 7)	64.15 $\pm$ 6.31	

^a Student T test, BIC: Bone Implant Connection.

Fig. 1. a, b: Undecalcified histologic images of the control (a) and experimental (b) groups' implants (4X) (X: ten times magnification, methylene blue). The implant surface not connected to the bone (α); the implant surface connected to the bone (β); the total implant surface: E ; the BIC ratio (%): $E-\alpha(\beta)/E$.

improving the success rate of implants and preventing the associated complications, the effects of a non-specific COX inhibitor, namely DCS, on the extent of the BIC of titanium implants placed in the tibial bones of rats were histologically evaluated in the present study. The evaluation of the degree of osseointegration and the calculation of the extent of the BIC using histologic sections are widely performed in preclinical studies, as they are known to result in a high level of evidence.^{1,13–17} Since it is difficult to place dental implants in the alveolar bones of rats, the tibia was instead used for the implantations, which has previously been shown to be an acceptable approach.^{18,19}

DCS was selected for investigation in this study because it is extensively used as an analgesic. It has been reported to delay the bone healing process in various preclinical models of bone healing following fracture, osteotomy, surgical procedures, and even tooth extraction.^{15,20–22} In the current study, the DCS was administered in appropriate doses (according to the body weights of the rodents) for two weeks following a four-week period of osseointegration, as prior studies have indicated that 28 days are required to ensure successful implant osseointegration in rats.^{23,24} Although the average BIC ratio was higher in the control group, the difference between the two groups was not statistically significant. Thus, the results of this study demonstrated that DCS did not impair bone healing around the implant.

In previous studies, the oral administration of both selective and non-selective COX inhibitors for four weeks following a six-week period of osseointegration was found to significantly suppress bone ingrowth in rabbits, with the results being attributed to the COX-2 inhibitors in particular.¹⁴ However, in other studies, the use of a selective COX-2 inhibitor for a short period of time was not found to impair bone ingrowth, although the effects on the bone were more intense when the drug was used for a longer period of time.¹⁶ The short-term use of NSAIDs is known to be safe.²⁵ Ribeiro et al.⁶ studied the effects of a selective COX-2 inhibitor, namely meloxicam (at a daily dosage of 0.2 mg/kg for five days), on bone healing around titanium implants in rats, and they concluded that continuous usage of the drug reduced the BIC, bone area, and bone density. Yet, in a study performed by Pablos et al. DCS was reported to inhibit peri-implant bone healing and to reduce the BIC, whereas meloxicam (3 mg/kg daily for 60 days—a 15-fold higher dose than the maximum recommended daily dosage) was not found to exert a negative effect.^{26,27} The dosages of the drugs used in such studies should be equivalent to the maximum daily dosages recommended for humans. The discrepancy in the findings between the aforementioned two studies can be attributed to the differences in the dosages. Winnett et al.²⁸ recently performed a retrospective study, and they concluded that dental implant osseointegration might be

negatively affected by the perioperative use of NSAIDs.

Although the results of this study conflict with those of the above-mentioned studies, support for the present findings does exist.^{6,14,26,28} For example, the administration of ibuprofen for a week in order to control postoperative pain was not observed to significantly affect the marginal bone around dental implants during the early healing period in a randomized clinical trial.²⁹ Further, Cai et al. investigated the short- and long-term effects of the administration of parecoxib and DCS on the osseointegration of dental implants in the calvarial bone.³⁰ They demonstrated that the administration of appropriate doses of DCS and parecoxib did not negatively affect osseointegration and bone healing in either the short or long term. In a recent study conducted by Salduz et al.,³¹ the effects of an eight-week postoperative course of 5 mg/kg/day of DCS and 3 mg/kg/day of celecoxib on osseointegration were investigated. The NSAIDs were not found to have a negative effect on either the extent of the BIC or osseointegration. Reddy et al.³² administered 100 mg of flurbiprofen twice daily for three months following implant placement, and they found that the bone density around the titanium implant increased. Flurbiprofen has also been found to reduce the amount of peri-implant bone loss in cases of induced implantitis.³³

Many studies have suggested COX-2 to be essential in relation to bone fracture healing, normal bone formation, and osseointegration, while COX-2-selective NSAIDs have been reported to significantly inhibit bone healing.^{17,34,35} It has been determined that COX-2, although not COX-1, is necessary for bone repair. COX-2 is also thought to co-ordinate a number of the transcription factors, including core-binding factor alpha 1 (Cbfa1) and osterix, involved in osteoblast differentiation and bone formation.³⁵ Cbfa1 and bone morphogenetic protein-2 (BMP-2) stimulate osteoblast differentiation by acting synergistically.³⁶ COX-2 enhances the production of prostaglandin E2 (PGE2), which is believed to stimulate BMPs as well as to augment the expression of Cbfa1 and osterix.

It has previously been demonstrated that human mesenchymal stem cells express COX-2, while the inhibition of PGE2 synthesis has been shown to reduce the expression of BMP-2. Such findings indicate that PGE2, which is produced by COX-2, is necessary for the expression of BMP-2. As a result, selective COX-2 inhibitors impair bone healing through inhibiting three processes: (i) the differentiation of mesenchymal cells into osteoblasts, (ii) the activity of those transcription factors that are essential to osteoblastogenesis, and (iii) COX-2 induction by BMPs.^{8,37} The decrease in osteoblastogenesis and bone formation that results from these mechanisms represents the most likely explanation of the negative findings reported in prior studies. The conflicting findings

of other studies can be explained by variations in the timings, durations, and doses of the different drugs used. The fact that DCS, as a non-selective COX inhibitor, was used in the present study can assist with understanding the earlier results. More reliable results might have been obtained if sand-blasted and acid-etched surface implants were used, as it is generally accepted that surface treatments are efficacious in terms of improving bone healing and augmenting the degree of osseointegration.^{38,39}

The present study did have a number of limitations. The administration of the DCS after four weeks of osseointegration was one such limitation because, within this short period of time, it was not possible to accurately evaluate a situation in which NSAIDs were used by patients on a daily basis to manage a chronic disease. A second limitation is that the molecular mechanisms underlying the association between the investigated NSAID and bone tissue metabolism could not be fully explained due to the method used in this study. Although animal studies are important for research and development in dentistry the final limitation concerns the fact that long bones such as the tibia and femur, when compared with the jaw bones (mandible–maxilla), have different osteogenic properties and, therefore, may respond differently to the application of NSAIDs.

5. Conclusion

Neither osseointegration nor bone healing were shown to be impaired in rat tibias following the two-week administration of a non-specific COX inhibitor, namely DCS, in the present study. Large-scale clinical studies, in which the long-term administration of NSAIDs is evaluated, are required to determine the clinical relevance and generalizability of the present results.

Funding

There is no funding.

Declaration of competing interest

The authors declare there is no conflict of interest.

Acknowledgement

The authors wish to thanks Implanse Dental Implant System, AGS Medical Corporation, Istanbul, Turkey for the providing the implants.

References

- Dundar S, Yaman F, Saybak A, et al. Evaluation of effects of topical melatonin application on osseointegration of dental implant: an experimental study. *J Oral Implantol*. 2016;42(5):386–389.
- American Academy of Periodontology. The American Academy of Periodontology Task Force on Peri-Implantitis. Periimplant mucositis and peri-implantitis: a current understanding of their diagnoses and clinical implications. *J Periodontol*. 2013;84(4):436–443.
- AAP. *Glossary of Periodontal Terms*. fourth ed. Chicago, IL: The American Academy of Periodontology; 2001:28.
- Fu JH, Bashutski JD, Al-Hezaimi K, Wang HL. Statins, glucocorticoids, and non-steroidal anti-inflammatory drugs: their influence on implant healing. *Implant Dent*. 2012;21(5):362–367.
- Goldkind L, Laine L. A systematic review of NSAIDs withdrawn from the market due to hepatotoxicity: lessons learned from the bromfenac experience. *Pharmacoevidenciol Drug Saf*. 2006;15:213–220.
- Ribeiro FV, Cesar-Neto JB, Nociti FH, et al. Selective cyclooxygenase-2 inhibitor may impair bone healing around titanium implants in rats. *J Periodontol*. 2006;77:1731–1735.
- Radi ZA, Khan NK. Effects of cyclooxygenase inhibition on bone, tendon, and ligament healing. *Inflamm Res*. 2005;54:358–366.
- Arikawa T, Omura K, Morita I. Regulation of bone morphogenetic protein-2 expression by endogenous prostaglandin E2 in human mesenchymal stem cells. *J Cell Physiol*. 2004;200:400–406.
- Lipsky PE. Role of cyclooxygenase-1 and -2 in health and disease. *Am J Orthop*. 1999;28:8–12.
- Pilbeam CC, Harrison JR, Raisz LG. Prostaglandins and bone metabolism. In: Bilezikian JP, Raisz LG, Rodan GA, eds. *Principles of Bone Biology*. New York: Academic Press; 2002.
- Sato Y, Arai N, Negishi A, Ohya K. Expression of cyclooxygenase genes and involvement of endogenous prostaglandin during osteogenesis in the rat tibial bone marrow cavity. *J Med Dent Sci*. 1997;44:81–92.
- Brideau C, Kargman S, Liu S, et al. A human whole blood assay for clinical evaluation of biochemical efficacy of cyclooxygenase inhibitors. *Inflamm Res*. 1996;45:68–74.
- Altman RD, Latta LL, Keer R, Renfree K, Hornicek FJ, Banovac K. Effect of non-steroidal antiinflammatory drugs on fracture healing: a laboratory study in rats. *J Orthop Trauma*. 1995;9:392–400.
- Goodman S, Ma T, Trindade M, Ikenoue T, et al. COX-2 selective NSAID decreases bone ingrowth in vivo. *J Orthop Res*. 2002;20:1164–1169.
- Beck A, Krishnak G, Sorg T, et al. Influence of diclofenac (group of nonsteroidal anti-inflammatory drugs) on fracture healing. *Arch Orthop Trauma Surg*. 2003;123:327–332.
- Goodman S, Ma T, Mitsunaga L, Miyazaki K, Genovese MC, Smith RL. Temporal effects of a COX-2 selective NSAID on bone ingrowth. *J Biomed Mater Res*. 2005;72:279–287.
- Chikazu D, Tomizuka K, Ogasawara T, et al. Cyclooxygenase-2 activity is essential for the osseointegration of dental implants. *Int J Oral Maxillofac Surg*. 2007;36:441–446.
- Jung UW, Kim S, Lee IK, Kim MS, Lee JS, Kim HJ. Secondary stability of micro-thickness hydroxyapatite-coated dental implants installed without primary stability in dogs. *Clin Oral Implants Res*. 2014;25(10):1169–1174.
- Shirotu T, Tashiro M, Ohno K, Yamaguchi A. Effect of intermittent parathyroid hormone (1–34) treatment on the bone response after placement of titanium implants into the tibia of ovariectomized rats. *J Oral Maxillofac Surg*. 2003;61:471–480.
- Yugoshi LI, Sala MA, Brentegani LG, Lamaso Carvalho TL. Histometric study of socket healing after tooth extraction in rats treated with diclofenac. *Braz Dent J*. 2002;13:92–96.
- Gerstenfeld LC, Thiede M, Seibert K, et al. Differential inhibition of fracture healing by non-selective and cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs. *J Orthop Res*. 2003;21:670–675.
- Sen C, Erdem M, Gunes T, Koseoglu D, Filiz NO. Effects of diclofenac and tenoxicam on distraction osteogenesis. *Arch Orthop Trauma Surg*. 2007;127:153–159.
- Krishnak GD, Augat P, Blakytyn R, et al. The non-steroidal anti-inflammatory drug diclofenac reduces appearance of osteoblasts in bone defect healing in rats. *Arch Orthop Trauma Surg*. 2007;127:453–458.
- Takeshita F, Murai K, Iyama S, et al. Uncontrolled diabetes hinders bone formation around titanium implants in rat tibiae. A light and fluorescent microscopy, and image processing study. *J Periodontol*. 1998;69:314–320.
- Utvag SE, Fuskevåg OM, Shegarfi H, Reikeras O. Short-term treatment with cox-2 inhibitors does not impair fracture healing. *J Invest Surg*. 2010;23:257–261.
- Pablos AB, Ramalho SA, Konig B, Jr Furuse C, de Araujo VC, Cury PR. Effect of meloxicam and diclofenac sodium on periimplant bone healing in rats. *J Periodontol*. 2008;79:300–306.
- Busch U, Schmid J, Heinzl G, et al. Pharmacokinetics of meloxicam in animals and the relevance to humans. *Drug Metab Dispos*. 1998;26:576–584.
- Winnert B, Tenenbaum HC, Ganss B, Joststad A. Perioperative use of non-steroidal anti-inflammatory drugs might impair dental implant osseointegration. *Clin Oral Implants Res*. 2016;27:1–7.
- Alissa R, Sakka S, Oliver R, et al. Influence of ibuprofen on bone healing around dental implants: a randomised double-blind placebo-controlled clinical study. *Eur J Oral Implant*. 2009;2:185–199.
- Cai WX, Ma L, Zheng LW, et al. Influence of non-steroidal antiinflammatory drugs (NSAIDs) on osseointegration of dental implants in rabbit calvaria. *Clin Oral Implants Res*. 2015;26:478–483.
- Saldaz A, Dikici F, Kilicoglu OI, et al. Effects of NSAIDs and hydroxyapatite coating on osseointegration: biomechanical and histological study on rabbits. *J Orthop Surg*. 2017;25:1–10.
- Reddy MS, Jeffcoat MK, Richardson RC. Assessment of adjunctive flurbiprofen therapy in root-form implant healing with digital subtraction radiography. *J Oral Implantol*. 1990;16:272–276.
- Weber HP, Fiorellini JP, Paquette DW, Howell TH, Williams RC. Inhibition of peri-implant bone loss with the nonsteroidal anti-inflammatory drug flurbiprofen in beagle dogs. A preliminary study. *Clin Oral Implants Res*. 1994;5:148–153.
- Simon AM, Manigrasso MB, O'Connor JP. Cyclooxygenase 2 function is essential for bone fracture healing. *J Bone Miner Res*. 2002;17:963–976.
- Zhang X, Schwarz EM, Young DA, Puzas JE, Rosier RN, O'Keefe RJ. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. *J Clin Invest*. 2002;109:1405–1415.
- Yang S, Wei D, Wang D, Phiphilai M, Krebsbach PH, Franceschi RT. In vitro and in vivo synergistic interactions between the Runx2/Cbfa1 transcription factor and bone morphogenetic protein-2 in stimulating osteoblast differentiation. *J Bone Miner Res*. 2003;18:705–715.
- Chikazu D, Li X, Kawaguchi H, et al. Bone morphogenetic protein 2 induces cyclooxygenase 2 in osteoblasts via a Cbfa1 binding site: role in effects of bone morphogenetic protein 2 in vitro and in vivo. *J Bone Miner Res*. 2002;17:1430–1440.
- Wennerberg A, Albrektsson T. On implant surfaces: a review of current knowledge and opinions. *Int J Oral Maxillofac Implants*. 2010;25:63–74.
- Warren P, Chaffee N, Felton DA, Cooper LF. A retrospective radiographic analysis of bone loss following placement of TiO2 grit-blasted implants in the posterior maxilla and mandible. *Int J Oral Maxillofac Implants*. 2002;17:399–404.