RESEARCH ARTICLE

F.U. Med.J.Health.Sci.
2023; 37 (3): 243 - 248
http://www.fusabil.org

Evaluation of the Effects of the Systemic Vitamin C Supplementation on Peri-Implant Bone Regeneration and Osseointegration: An Experimental Study *

Merve YILMAZ BOZOĞLAN [1, a]
Serkan DÜNDAR [2, b]
Alihan BOZOĞLAN [2, c]
Tuba TALO YILDIRIM [2, d]
Erhan Cahit ÖZCAN [3, e]
Mustafa KIRTAY [4, f]
Can Ayhan KAYA [5, g]
Osman HABEK [6, h]

[1] Fırat University, Faculty of Medicine, Department of Pharmacology, Elazig, TURKIYE

[2] Fırat University, Faculty of Dentistry, Department of Periodontics, Elazig, TURKIYE

[3] Fırat Üniversitesi, Faculty of Medicine, Department of Plastic, Reconstructive and Aesthetic Surgery, Elazig, TURKIYE

[4] Private Practice Physician, Oral and Maxillofacial Surgery, Ontario, KANADA

[5] Dicle University, Diyarbakır Agricultural College, Dairy and Livestock Program, Diyarbakir, TURKIYE

[6] Private Practice Physician, Oral and Maxillofacial Surgery, Sanliurfa, TURKIYE

[a] ORCİD: 0000-0001-6058-4579
[b] ORCİD: 0000-0003-3944-1957
[c] ORCİD: 0000-0003-3420-5816
[d] ORCİD: 0000-0002-7577-5703
[e] ORCİD: 0000-0001-6513-0226
[f] ORCİD: 0000-0003-2568-7470
[g] ORCİD: 0000-0001-8226-7603
[h] ORCİD: 0000-0002-2276-8884

Received : 14.12.2022
Accepted : 09.08.2023

Yazışma Adresi
Correspondence

Serkan DÜNDAR
Fırat University,
Faculty of Dentistry,
Department of Periodontics,
Elazig, TURKIYE

sdundar@firat.edu.tr

Guided bone regeneration (GBR) is a dental surgical prosedure used to stimulate bone growth in tissue defects adjacent to dental implants. This study purposed to evaluate the effect of dietary supplementation of Vitamin C (Vit C) on the osseointegration and periimplant bone regeneration after implant insertion surgery with GBR. Thirthy Sprague Dawley rats were enrolled in the study and they were divided into 3 groups as control, Vit C 1 and Vit C 2. Two different doses (5 mg/kg and 10 mg/kg) of Vit C were performed to the Vit C 1 and Vit C 2 groups, respectively. Ossoeintegration and periimplant bone tissue regeneration analysed by reverse torque method. No statistically significant difference was observed in the biomechanical reverse torque analysis in the implants of the groups. Although osseointegration was higher numerically in the Vit C 1 group, this was not statistically significant. Vit C supplementation did not increase the osseointegration with GBR (P>0.05) (P:0.920). If the numerical increase in our findings were significant, it may be considered to recommend Vit C supplementation to patients scheduled for implant surgery

Key Words: *Osseointegration, bone implant connection, guided bone regeneration, vitamin c, vitamin supplementation*

Sistemik C Vitamini Takviyesinin Peri-İmplant Kemik Rejenerasyonu ve Osseointegrasyon Üzerindeki Etkilerinin Değerlendirilmesi: Deneysel Bir Çalışma

Yönlendirilmiş kemik rejenerasyonu (YDR), dental implant çevresi kemik doku defektlerinde kemik gelişimini arttırmak için kullanılan bir ağız cerrahisi prosedürüdür. Bu çalışma, YDR ile implant yerleştirme cerrahisinden sonra diyetle C Vitamini (Vit C) takviyesinin osseointegrasyon ve periimplant kemik rejenerasyonu üzerindeki etkisini değerlendirmeyi amaçladı. Çalışmaya 30 Sprague Dawley rat dahil edildi ve denekler kontrol, Vit C 1 ve Vit C 2 olarak eşit 3 gruba ayrıldı. Vit C uygulanan deney gruplarına iki farklı doz (5 mg/kg ve 10 mg/kg) Vit C uygulandı. Osseointegrasyon ve implant çevresi rejenerasyon tersine tork analiz yöntemi ile analiz edildi. Gruplar arasında biyomekanik ters tork analizinde istatistiksel olarak anlamlı fark gözlenmedi. Vit C 1 grubunda osseointegrasyon sayısal olarak daha yüksek olmasına rağmen istatistiksel olarak anlamlı değildi (P>0.05) (P:0.920). C vitamini takviyesi, GBR ile osseointegrasyonu artırmadı. Bulgularımızdaki istatistiksel olarak gruplar arasında fark olmasa da sayısal artışın anlamlı olması implant cerrahisi planlanan hastalara C vitamini takviyesinin önerilmesini düşünülebilir.

Anahtar Kelimeler**:** *Ossoeintegrasyon, kemik implant kaynaşması, yönlendirilmiş kemik rejenerasyonu, vitamin c, vitamin supplementation*

Introduction

Titanium is an excellent example of mechanical strength with being stronger and lighter than steel. It has a very high corrosion resistance under a biological environment and the ability to develop a dynamic oxide layer on the surface (1-3). Dental implants are considered as the gold standard treatment to replace missing natural teeth. In addition to solving functional problems in partial or complete edentulism, oral rehabilitation with implant supported prosthetic treatment also provides solutions to aesthetic problems and possible accompanying psychological situations (4). The titanium implants must heal by making direct contact with the bone tissue without the interposition of soft tissue after implant surgery (5). This connection is called osseointegration, and it can also be named as the mechanical contact of bone cells at a cellular level by penetrating the rough surface of the implant. Osseointegration and GBR actually depend on bone healing process. The stronger the osseointegration, the more desirable functions such as chewing, crushing, and tearing will be present. The physician's experience, training, expertise, and manual ability; bone quantity and quality

* This study was supported by Firat University Scientific Project Foundation Department with the project number DHF.21.02.

of the patient; oral care and general diseases of the patient; smoking; presence of gum disease in the patient before dental implant loading are the most important factors affecting implant success (6). However, nowadays, in addition to all these, it is thought that the nutritional status and nutritional content of the patient may be very important in implant loading, osseointegration and the ongoing process.

Vitamin C (Vit C), a water-soluble vitamin, has a six-carbon lactone in structure. Some mammals synthesize Vit C from glucose in their bodies. As there is no L-gulonolactone oxidase enzyme in humans, ascorbic acid synthesis does not occur. Therefore, the necessary amount of Vit C for the human body must be met fully through daily intake (7). The daily recommended amount varies between men and women, but is approximately 70-90 mg/day and it increases in pregnancy and lactation. Although Vit C is generally known as an antioxidant and oxidative stress scavenger due to its protective effects in cardiovascular diseases, obesity and neurodegenerative diseases, cancers and inflammation processes. It was discovered that the first positive effect of Vit C on the cardiovascular system was on endothelial dysfunction. The results obtained from this discovery have provided other positive effects on the cardiovascular system in association to coronary artery disease and related deaths in patients with low Vit C levels (8). On the other hand it has been observed that patients with cancers such as gastric cancer generally have lower levels of Vit C than healthy adults. Vit C also decreases oxidative stress on the living tissue (9). The role of Vit C in inflammation can be summarized as the differentiation and function of immune cells and epithelial barrier cells. Low levels of Vit C have been noted in inflammation, just as in patients with cancer or cardiovascular diseases (10). Moreover Vit C stimulates the synthesis of collagen, which is the most important component of the extracellular bone matrix, and the formation of osteoblasts, which are bone-forming cells. It also scavenges free radicals that are harmful to bone health and reduces oxidative stress. Thus, it is thought to protect bones against osteoporosis by preventing bone resorption (11). Vit C, whose positive effects on bone tissue have been determined, has also taken its place in dentistry and dental implant studies, which have become very popular recently. Platelet-rich fibrin with ascorbic acid has been reported to enhance gingival tissue gain and fill radiographic defect, in periodontitis patients (12). Cho et al. (13) reported that guided bone regeneration (GBR) was used to stimulate bone growth in tissue defects adjacent to dental implants. The GBR adjacent to the implant triggers active bone modeling for the rapid filling of new bone tissue at the defect area in the early post-implantation period. Another effect of Vit C that will increase implant bone connection, is its positive effect on wound healing. Ascorbic acid stimulates wound closure by collagen turnover and by manipulating the state of the inflammation. In a study comparing ascorbic acid and zinc, it was found that Vit C was 4 times stronger than zinc oxide in wound closure (14). Li et al. reported that Vit C supplementation improved postoperative recovery following dental implant surgery in patients with chronic periodontitis and patients treated with GBR (15). This study aimed to evaluate the effect of dietary supplementation of Vit C on the osseointegration after implant insertion surgery with GBR.

Materials and Methods

Research and Publication Ethics: Approval for the study was granted by the university's Fırat University Animal Experimental Ethics Council with number 426281 (30.11.2020). The ARRIVE guidelines was strictly followed during this experimental study.

Animals and Study Design: The experiments have been carried out in accordance with the Declaration of Helsinki. 30 female Sprague Dawley rats which were aged 3 to 5 months were obtained from the Fırat University Experimental Research Center (Elazig, Turkiye). The mean bodyweight of the rats was measured approximately 280 to 300 gr. The rats were held in cages with 12-hour light and 12-hour dark cycle and a standard temperature 24±2 C. They were fed standard chow and water ad libitum. Vaginal swabs were taken for standardization and all rats were in the same oestrus period.

The number of rats required for the experimental setup was determined by the power analysis; It was calculated that there should be at least 8 rats in each group when the deviation was 8%, type 1 error (α) was 0.05 and type 2 error (β) (Power=0.80). The study was started with 10 rats in each group in case the rats died during the surgical procedures and during the experimental setup.

Control graft group (n=10): As in all groups, the right tibial metaphysis corticocancellous part was reached surgically, in the control group. Bone cavities were opened with proper surgical drills under steril serum perfusion. And titanium implants with a diameter of 2.5 mm diameter and the length of 4 mm were loaded in cavities. The bone graft (Cerabone, Botiss Biomaterials, Zossen Germany) was placed in the bone defect that can be defined as the area correspponding to 2 mm of the implant length in the neck region. No other surgical intervention or drug administration were applied to the control group rats until the end of 8 weeks when the procedure was completed.

Vit C dose 1 graft group (n=10): The same surgical procedure was performed as applied to the control graft group. However, 5 mg/kg of Vit C was administered during the 8 weeks period (three days a week) by oral gavage (15).

Vit C dose 2 graft group (n=10): The same surgical procedure was performed as applied to the control graft group. This time, unlike dose 1 group, 10 mg/kg Vit C was administered. At the end of 8 weeks, all rats were sacrificed and the experiment was terminated. Then the implants and surrounding bone tissue were removed gently and subjected to biomechanical analyse tests that evaluate bone implant connection and bone regeneration (15).

Surgical Procedures: All surgical procedures were performed in environments complying with sterility conditions and under general anesthesia. The rats have fasted for 8 hours before anesthesia. General anesthesia was achieved with intramuscular injections of 40 mg/kg ketamine hydrochloride (Ketasol®, Richter Pharma, Austria) and 5 mg/kg xylazine hydrochloride (Rompun®, Bayer, Germany). Mepivacaine hydrochloride (0.3 mL/kg, scandicaine epinephrine 1:100,000 to 2%; Septodont, France) infiltration was used to reduce hemostasis at the wound site. The surgical area to be operated was shaved. then it was stained with povidone iodide for sterilization. After making an incision of approximately 1.5 cm over the tibial crest with a No. 15 scalpel, a periosteal elevator was used to reach the proximal tibia. Cavities were opened in the right tibial metaphyseal corticocancellous bones of the rats (Figure 1). Half of the length of the cavity was opened with a diameter of 2.5 mm, while the upper half was opened with a diameter of 4.5 mm. Titanium implants with a diameter of 2.5 mm and a length of 4 mm (Implance Dental Implant System, AGS Medical Corporation, Istanbul, Turkiye) were placed in these cavities. And a bone graft was placed into the bone defect corresponding to 2 mm of the implant length in the neck region. The surgical procedure was completed by closing the flaps using absorbable sutures (4/0 vicryl; Ethicon, Inc., Somerville, NJ, USA) for the soft tissues and monofilament sutures for the skin (nylon 4.0; Ethicon, Inc.) (2,3). In postoperative chemotherapy, antibiotics (50 mg/kg penicillin) and analgesics (0.1 mg/kg tramadol hydrochloride) were administered intramuscularly every 24 hours for three days. No additional application was made during the eight-week experiment period. During the postoperative period, the rats were followed daily for possible complications such as pain, separation, infection, restricted movement and weight loss. All rats were decapitated after an eight-week recovery period and the experiment was terminated. Implants were taken for biomechanical analysis together with the surrounding bone tissues.

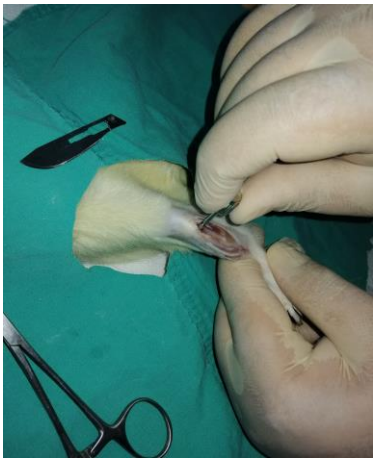

Figure 1. Surgical integration of the 2.5 mm diameter and 4 mm length titanium implants.

Biomechanical Analysis: The experimental groups were started with ten rats to be prepared for the possibility of rat loss during the surgical applications and experimental period. Two rats from the control group were excluded from the study because their implants did not fit properly. The implant parts taken together with the surrounding bone tissues were prepared to be tested as blocks and they were kept in 10% buffered formalin solution. All implants were placed in polymethylmethacrylate blocks immediately to prevent dehydration. A rotating apparatus was placed to measure the torque of the implants. An extraction force was applied manually, slowly and incrementally counterclockwise using a digital torque instrument (Mark 10, NY, USA) (Figure 2) (3). As soon as the dental implant returned to the bone socket, the procedure was terminated immediately. The highest torque force (Ncm) measured during the first rotation of the implant in the socket was automatically recorded.

Figure 2. Biomechanical (Mark 10, NY, USA); reverse torque analysis, of the implants.

Statistical Analysis: Statistical analysis was performed using IBM SPSS Statistics (Version 22.0). The appropriateness of the normal distribution of the parameters was assessed by the Kolmogorov–Smirnov test. A one-way analysis of variance (one-way ANOVA) was performed for the parameters, showing a normal distribution. Tukey's Honestly Significant Difference test was used to detect the group causing the difference. Continuous data were presented as mean ± standard deviation values, and numerical measurements were presented as median, minimum, and maximum values. A statistical significance level of 95% ($P<0.05$) was adopted.

Results

In the biomechanical reverse torque analysis, no statistically significant difference was observed between the groups ($P>0.05$), (P:0.920). As shown in Table 1, although osseointegration was higher in the Vit C dose

1 graft group compared to the control and Vit C dose 2 group numerically, this difference did not reach statistical significance (P>0.05) (P:0.920).

Table 1. Biomechanical reverse torque analysis in of the groups after guided bone regeneration

Groups	N	Mean (N±cm)	Std. Deviation	P*
Vit C dosage 1	10	5.98	2.26	
Vit C dosage 2	10	5.66	1.58	0.920 (>0.05)
Control	8	5.66	2.18	

Vit C dosage 1 and 2 groups were applied 5 mg/kg and 10 mg/kg Vit C respectively. *One Way Anova

Discussion

To our knowledge, the effect of Vit C on dental implant osseointegration in rat living bone tissue undergoing GBR was examined for the first time in this study. On the other hand, few molecular dynamics simulations, animal experimental studies or cell culture studies have shown that Vit C improves osseointegration and has positive effects on bone tissue. In this sense, clinical observation studies are also available. We reviewed these research designs when calculating the dose of Vit C to be administered to the rats in our study. For example, we noted that the synthesis of collagen type I, osteonectin and osteocalcin levels increased with 200 µg/ml of Vit C, in an in-vitro study (16). Although various factors such as gender, age, smoking, pregnancy and breastfeeding can change the amount of Vit C required, it can be said that the amount to be taken daily is roughly 75-105 mg/day (17). Moreover, studies suggest that single oral doses higher than 200 mg are characterized by relatively low bioavailability, suggesting that such high doses should rather be divided into several subdoses (18). Taking this pharmacokinetic information into account, we administered 5 mg of Vit C, which we calculated as an adult human equivalent dose, to rats, since no other rat studies were available.

The experience of the physician and the quality of the implant are often not sufficient for perfect osseointegration. Nastri et al. (19) stated that for perfect osseointegration, the immunological status of the host and its nutritional content and balance should be taken into account. It is noteworthy that the effects of daily nutrients, including vitamins and minerals, may be more than expected, and more studies are being conducted in this area. Because of its direct effects on bone metabolism, vitamin D has been the most researched vitamin in studies. It is known that vitamin D deficiency slows down implant osseointegration and increases the risk of graft infection (20). Liu et al. reported that vitamin D supplementation has increased the fixation of titanium implants in the case of chronic renal failure, which has negative effects on bone metabolism (21). In another experimental study, the effect of vitamin supplementation was measured by using immediate implants instead of extracted premolar teeth of dogs. It was remarkable that vitamin D supplementation in these dogs without vitamin D deficiencies reduced crestal bone loss and increased osseointegration (22). In one of the above-mentioned studies, Vit C supplementation in addition to vitamin D, increased the success of dental implant surgery in patients with vitamin D deficiency (19). However, although the literature evaluating the effects of Vit C on bone tissue has been reached, there are very few studies that have observed its effects on dental implants and osseointegration. A study of Vit C on the gene level showed that Vit C improves trabecular bone formation by influencing the bone matrix gene expression of osteoblasts (23). In a study comparing the data of the fifth lumbar vertebra and femur in ovarectomized rats, it was found that Vit C increased bone density (24). Malmir et al. conducted a meta-analysis on clinical researches (11). They stated that higher dietary Vit C intake resulted in a lower risk of hip fracture and osteoporosis, as well as a higher bone mineral density in the femoral neck and lumbar spine. Vit C contributes to the normal function of bones and teeth by enhancing osteoblastogenesis and inhibiting osteoclastogenesis through Wnt/β-catenin signaling. In this study, it has been shown for the first time that Vit C improves bone regeneration and teeth health (25). Thereafter, the European Food Safety Authority's opinion of Vit C was shaped by its contribution to the normal function of bones and teeth. All these findings and opinions of the authorities support our results that a positive effect was observed at the 5 mg/kg dose of Vit C. There is no definite indication for the use of Vit C in wound healing. However, supplementation with Vit C for 10 days after extraction of premolar teeth has been shown to improve wound healing (26). In a cell culture study by Li et al., it was stated that gelatins doubly modified with Vit C and β-glycerophosphate salt can be scaffolds for versatile bone tissue engineering (27). With its effectiveness in both bone tissue and non-bone tissue, Vit C seems to be essential for osseointegration following dental implant surgery to be at the desired level. These effects were confirmed by a randomized controlled trial, in which 128 patients with chronic periodontitis were treated with Vit C after dental implant surgery with bone grafts or GBR like in our study. It was observed that the wound healing of the patients who took vitamin C supplements for 14 days after the surgery were better than the patients who did not. Another conclusion of the same study is that vitamin C supplementation provides a more significant improvement especially in patients with chronic periodontitis who underwent implant surgery (15). The most recent systematic review that was published in 2022 stated that Vit C accelerated bone healing without causing any side effects (28). This review also supports the more remarkable result of our study, that with an application dose above the plateau (10 mg), the osseointegration values remained almost the same as those of the control group without causing any advers effects in the reverse torque.

Reverse torque analysis is used in in vivo and in vitro research to assess osseointegration. The reverse torque analysis method cannot be used clinically. Reverse torqueing of the implant in the bone tissue into

which it is placed provides a biomechanical measurement of the force required to break the bone-implant connection. It is an objective method for evaluating bone-implant attachment. Reverse torque analysis is a method that allows the evaluation of the interaction of all bone tissue around the titanium implant with the implant surface. A very thin section is required for histological analysis. In our study, reverse torque test was used to evaluate the effect of different postoperative vitamin C doses on osseointegration according to the literature. Although numerical differences were detected between the groups, no statistical difference was detected (29).

Research on the role of Vit C supplementation in patients with dental implants continues with interest. It is not difficult to predict that Vit C, which is essential for the maturation of collagen and the normal function of bone tissue, is a virgin area in dentistry studies and will be examined as much as vitamin D in the future. Based on the limited results of this study, the difference in ossointegration levels in the experimental groups at the numerical level, although not statistically, is promising in terms of the Vit C and bone tissue regeneration relationship. Further studies are needed to examine the relationship between osseointegration and Vit C.

Acknowledgement

The authors wish to thanks Implance Dental Implant System, AGS Medical Corporation, Istanbul, Turkiye for providing the titanium implants.

Competing interests: The authors declare there is no competing interests. The authors do not have any financial interests, either directly or indirectly, in the products or information listed in the paper.

References

1. Bozoglan A, Dundar S. Comparison of osseointegration of Ti-Al_6V_4 and Ti-Al_6Nb_7 implants: An experimental study. J Oral Biol Craniofac Res 2021; 11: 624-627.
2. Dundar S, Yaman F, Bozoglan A, et al. Comparison of osseointegration of five different surfaced titanium implants. J Craniofac Surg 2018; 29: 1991-1995.
3. Gunes N, Gul M, Dundar S, et al. Biomechanical evaluation of implant osseointegration after guided bone regeneration with different bone grafts. J Craniofac Surg 2021; 32: 1545-1548.
4. Naemi R, Barikani HR, Shahmoradi L. Dental implant quality registries and databases: A systematic review. J Educ Health Promot 2021; 10: 214.
5. Ozcan EC, Gul M, Dundar S, et al. Effects of local application of the ankaferd blood stopper on osseointegration in three different surface titanium implants. J Oral Biol Craniofac Res 2021; 11: 524-528.
6. Goiato MC, dos Santos DM, Santiago JF Jr, et al. Longevity of dental implants in type IV bone: A systematic review. Int J Oral Maxillofac Surg 2014; 43: 1108-1116.
7. Granger M, Eck P. Dietary vitamin C in human health. Adv Food Nutr Res 2018; 83: 281-310.
8. Moser MA, Chun OK. Vitamin C and heart health: A review based on findings from epidemiologic studies. Int J Mol Sci 2016; 17: 1328.
9. Toh JWT, Wilson RB. Pathways of gastric carcinogenesis, helicobacter pylori virulence and interactions with antioxidant systems, vitamin C and phytochemicals. Int J Mol Sci 2020; 21: 6451.
10. Padayatty SJ, Levine M. Vitamin C: the known and the unknown and goldilocks. Oral Dis 2016; 22: 463-493.
11. Malmir H, Shab-Bidar S, Djafarian K. Vitamin C intake in relation to bone mineral density and risk of hip fracture and osteoporosis: A systematic review and meta-analysis of observational studies. Br J Nutr 2018; 119: 847-858.
12. Elbehwashy MT, Hosny MM, Elfana A, et al. Clinical and radiographic effects of ascorbic acid-augmented platelet-rich fibrin versus platelet-rich fibrin alone in intra-osseous defects of stage-III periodontitis patients: A randomized controlled clinical trial. Clin Oral Investig 2021; 25: 6309-6319.
13. Cho KS, Choi SH, Han KH, et al. Alveolar bone formation at dental implant dehiscence defects following guided bone regeneration and xenogeneic freeze-dried demineralized bone matrix. Clin Oral Implants Res 1998; 9: 419-428.
14. Salgado RM, Cruz-Castañeda O, Elizondo-Vázquez F, et al. Maltodextrin/ascorbic acid stimulates wound closure by increasing collagen turnover and TGF-β1 expression in vitro and changing the stage of inflammation from chronic to acute in vivo. J Tissue Viability 2017; 26: 131-137.
15. Li X, Tang L, Lin YF, et al. Role of vitamin C in wound healing after dental implant surgery in patients treated with bone grafts and patients with chronic periodontitis. Clin Implant Dent Relat Res 2018; 20: 793-798.
16. Urban K, Höhling HJ, Lüttenberg B, et al. Biomineralisation Research Unit. An in vitro study of osteoblast vitality influenced by the vitamins C and E. Head Face Med 2012; 8: 25.
17. Cerullo G, Negro M, Parimbelli M, et al. The long history of vitamin C: From prevention of the common cold to potential aid in the treatment of COVID-19. Front Immunol 2020; 11: 574029.
18. Pawlowska E, Szczepanska J, Blasiak J. Pro- and antioxidant effects of vitamin C in cancer in correspondence to its dietary and pharmacological concentrations. Oxid Med Cell Longev 2019; 2019: 7286737.
19. Nastri L, Moretti A, Migliaccio S, et al. Do dietary supplements and nutraceuticals have effects on dental implant osseointegration? A scoping review. Nutrients 2020; 12: 268.
20. Choukroun J, Khoury G, Khoury F, et al. Two neglected biologic risk factors in bone grafting and implantology: High low-density lipoprotein cholesterol and low serum vitamin D. J Oral Implantol 2014; 40: 110-114.

21. Liu W, Zhang S, Zhao D, et al. Vitamin D supplementation enhances the fixation of titanium implants in chronic kidney disease mice. PLoS One 2014; 9:e95689.

22. Salomó-Coll O, Maté-Sánchez de Val JE, Ramírez-Fernandez MP, et al. Topical applications of vitamin D on implant surface for bone-to-implant contact enhance: A pilot study in dogs part II. Clin Oral Implants Res 2016; 27: 896-903.

23. Aghajanian P, Hall S, Wongworawat MD, et al. The roles and mechanisms of actions of vitamin C in bone: New developments. J Bone Miner Res 2015; 30: 1945-1955.

24. Deyhim F, Strong K, Deyhim N, et al. Vitamin C reverses bone loss in an osteopenic rat model of osteoporosis. Int J Vitam Nutr Res 2018; 88: 58-64.

25. Choi HK, Kim GJ, Yoo HS, et al. Vitamin C activates osteoblastogenesis and inhibits osteoclastogenesis via Wnt/β-Catenin/ATF4 signaling pathways. Nutrients 2019; 11: 506.

26. Pisalsitsakul N, Pinnoi C, Sutanthavibul N, et al. Taking 200 mg vitamin C three times per day improved extraction socket wound healing parameters: A Randomized clinical trial. Int J Dent 2022; 2022: 6437200.

27. Li D, Zhang K, Shi C, et al. Small molecules modified biomimetic gelatin/hydroxyapatite nanofibers constructing an ideal osteogenic microenvironment with significantly enhanced cranial bone formation. Int J Nanomedicine 2018; 13: 7167-7181.

28. Barrios Garay K, Toledano Serrabona J, Gay Escoda C, et al. Clinical effect of vitamin C supplementation on bone healing: A systematic review. Med Oral Patol Oral Cir Bucal 2022 ; 27: e205-e215.

29. Tekin S, Dundar S, Demirci F, et al. Biomechanical and biochemical evaluation of the effect of systemic application of omeprazole on the osseointegration of titanium implants. Int J Implant Dent 2021;7: 27.