Histologic and Micro-CT Findings by the Impact of Vitamin D on Osseointegration in the Ovariectomized Rat Maxilla: A Preliminary Study

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ABSTRACT

This study was aimed to determine the effect of vitamin D on bone formation around implant in the maxilla of osteoporotic rats. Fifteen female rats at 8 weeks-old were divided into three groups: 1) control group, sham-operated rats, 2) ovariectomized only group (OVX) and 3) ovariectomized and vitamin D-administered group (VitD). Eight weeks after the ovariectomy or sham operation, upper right molar was extracted, and an implant was placed at 4 weeks post-extraction. The vitamin D-administered experimental groups were given with 0.1 μg/kg/day via gavage while the OVX group and the control group received vehicle only. All rats in each group were sacrificed at 8 weeks and histologic and micro-computed tomographic (CT) evaluation were performed. According to the histologic observation, newly formed bone close to implant threads was mature with considerable quantity of bone marrow in the VitD group than the OVX group. In micro-CT findings, the VitD groups showed greater radiologic density of peri-implant bone close to implant threads than the OVX group. The results proposed that vitamin D could increase bone formation around implant. This might have clinical implications in dental implant treatment for patients with reduced bone formation capacity.

Key words : Bone formation, Dental implant, Osteoporosis, Ovariectomy, Vitamin D

INTRODUCTION

Clinical success of dental implant depends on the osseointegration which is defined as intimate contact between titanium to bone[1]. In order to provide a stable and functional dental restoration, there are several attempts to improve the osseointegration cascade by the modification of implant surface[2,3], administration of drug that influence bone metabolism and application of bioactive substance that stimulate the factors involved in bone healing[4,5]. Since poor bone quality and quantity at the implant recipient site is main cause of failures of implants due to lack of initial stability, it needs to be interested in substances that facilitate bone formation around implant[6-8].

Systemic diseases may disturb the healing process and potentially affect implants and tissues carrying the implants. Among these diseases, osteoporosis is the most common and a risk factor for compromised osseointegration[9]. Osteoporosis is a degenerative bone disease affecting postmenopausal women and individuals aged over 50 years. Estrogen deficiency after menopause and osteopenia following the aging process cause the reduced bone mass and bone quality. This leads to weakened bone strength and the risks of fractures that occur most commonly in the spine, wrist, and hip are increased[10].

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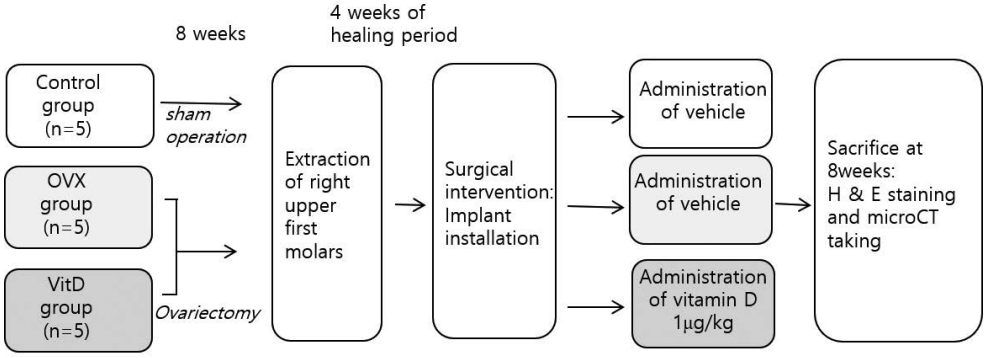

Figure 1. *Experimental design showing the allocation of animals and schedule for administration of vitamin D.*

Vitamin D is a hormone that affects the musculoskeletal system by maintaining normal blood levels of calcium and phosphorus. It is transformed into an active form, 1,25-dihyroxyvitamin D($1,25(OH)_2D$), by transported to the liver and doubly hydroxylated at the liver and kidney. This process was stimulated by parathyroid hormone, hypocalcemia, and hypophosphatasemia, but inhibited by calcium ion and $1,25(OH)_2D$[11,12]. Then, it regulates bone mineralization by activation of bone-forming osteoblasts and bone-resorbing osteoclastic cells. Therefore, vitamin D is also being widely used in the prevention of bone loss and osteoporosis-related fractures[13].

Recently, debate has been drawn to the efficacy of vitamin D supplementation on osseointegration of implants since low levels of vitamin D can cause deleterious effects on bone tissues[14,15]. In the experimental studies, administration of vitamin D has been shown to enhance bone-to-implant contact (BIC) as compared with controls[16,17]. However, negative result has also been reported indicating no substantially increased BIC[18-20].

Osteoporosis is a degenerative bone disease and causes the decrease of bone mass as well as bone quality[10]. Since osteoporosis negatively affects the balance of bone turnover and compromised regeneration capacity, it is thought to impair osseointegration of dental implants[9]. Meanwhile, in the observational study about serum vitamin D concentration of postmenopausal osteoporotic female, it was reported that vitamin D deficiency is very high in this population[21].

To date, there were few studies that investigated the effect of vitamin D on the osseointegration of dental implants using animal models and human[18,20,22,23]. Still, no study has performed on the effect of vitamin D supplement on osseointegration using an osteoporotic rat maxilla model. In this research, the authors hypothesized that vitamin D could improve the establishment of osseointegration of titanium implants. To verify this assumption, an implant was installed in the maxilla of ovariectomized rat and evaluated the impact of vitamin D by histologic observation and micro-computed tomography (micro-CT) assessment.

MATERIALS AND METHODS

1. Preparation of experimental animals

Fifteen female Sprague-Dawley rats at 8-week-old (average weight: 250~300 g, Nara Biotech, Pyeongtaek, Korea) were used in this study. Animals were raised at room temperature (25°C) with relative humidity (55%) and alternating light rhythms of 12 h. All rats were fed a commercial standard diet and water was freely available. They were randomly divided into three groups (n = 5, respectively): The control group contained rats that underwent a sham operation, the ovariectomy group (OVX) contained ovariectomized rats, the ovariectomized and vitamin D-administered group (VitD) contained ovariectomized and given with vitamin D (Fig 1). Ethical approval for animal handling and surgical procedures was obtained from the Institutional Animal Care and Use Committee (BSM 18-001).

2. Surgical procedures

After one week of acclimatization, bilateral ovariectomy to induce osteoporosis or sham-operation were performed under the inhalation anesthesia using 3% isoflurane (JW Medical Co., Seoul, Korea) on the rats according to group. Eight weeks later, the first molar of the right side of the maxilla was extracted to make the edentulous state under general anesthesia by the 30 mg/kg of zolazepam-tiletamine (Zoletil, Virbac

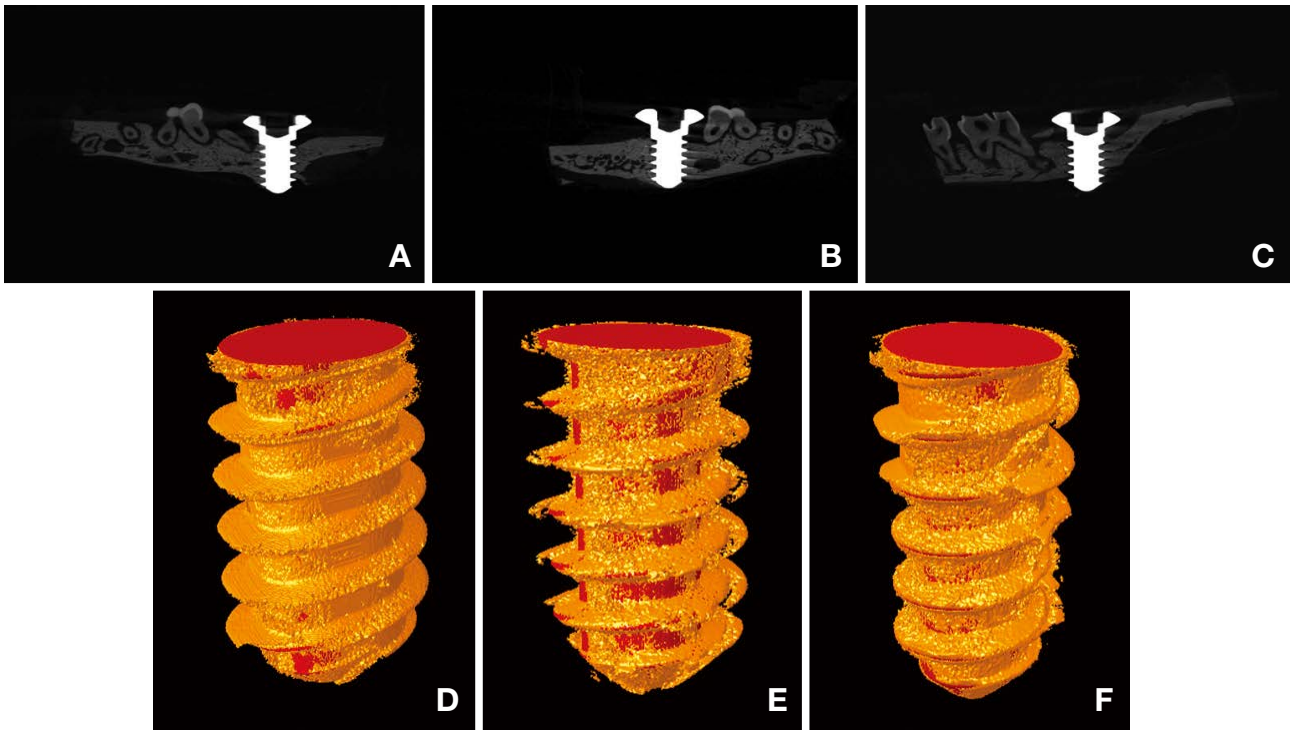

Figure 2. *2D micro-CT images (upper row) and 3D reconstructed images (lower row) of implant and the adjacent bone. Images of the sham-operated control group (A, D) indicate intimate bone to implant contact. Images of the OVX group (B, E) display defective bone and the compromised healing of bone around the implant and defective bone. The VitD group (C, F) shows relatively increased radio-opacity and favorable bone density around the implant. Red-color: titanium screw implant; yellow-color: appositional peri-implant bone.*

S.A., Carros, France) and 10 mg/kg of xylazine hydrochloride (Rompun, Bayer Korea, Seoul, Korea). Given four weeks of healing period, rats were put under general anesthesia again with zolazepam-tiletamine and xylazine, an implant receiving site was prepared by a pilot drill (1.0 mm in diameter) on the previously extracted socket of maxilla. Then, a titanium screw implant (Ti-Al6-V4, Gssem, Co. Seoul, Korea) (1.2 mm in diameter and 3 mm in length) was installed into the site.

3. Administration of vitamin D

Vitamin D (cholecalciferol, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in middle chain triglyceride (MCT) vehicle and administrated by oral gavage at 1 μg/kg/day from the day after implant placement. The same amount of MCT vehicle was administered to rats in the control group and the OVX group. After treatment with vitamin D or vehicle for 8 weeks, all rats were euthanized and the maxillary bone blocks with implant were harvested for histologic observation and micro-CT evaluation.

4. Micro-CT analysis

The bone blocks with screw was scanned on a micro-CT scanner (Skyscan 1173, Bruker MicroCT, Kontich, Belgium) in axial direction of the implant. Image pixel size of 5.33 μm was obtained at 130 kV and 45 μA with a 1.0 mm aluminum filter and a 5000 ms exposure time. The 3D images were reconstructed with Nrecon program (Bruker MicroCT, Belgium). The region of interest (ROI) included the trabecular compartment extending apically 2.5 mm and 0.3 mm outward from the implant perimeter.

5. Histologic evaluation

After micro-CT scan, bone specimens were fixed in 4% paraformaldehyde (Fischer Scientific, Columbus, OH, USA) for 24 hrs and demineralized with 10% EDTA. After demineralization for 4 weeks, the screw was removed carefully, and specimens were dehydrated and embedded with paraffin. For histological observation, sections were stained with hematoxylin and eosin (H & E) and were reviewed using an optical

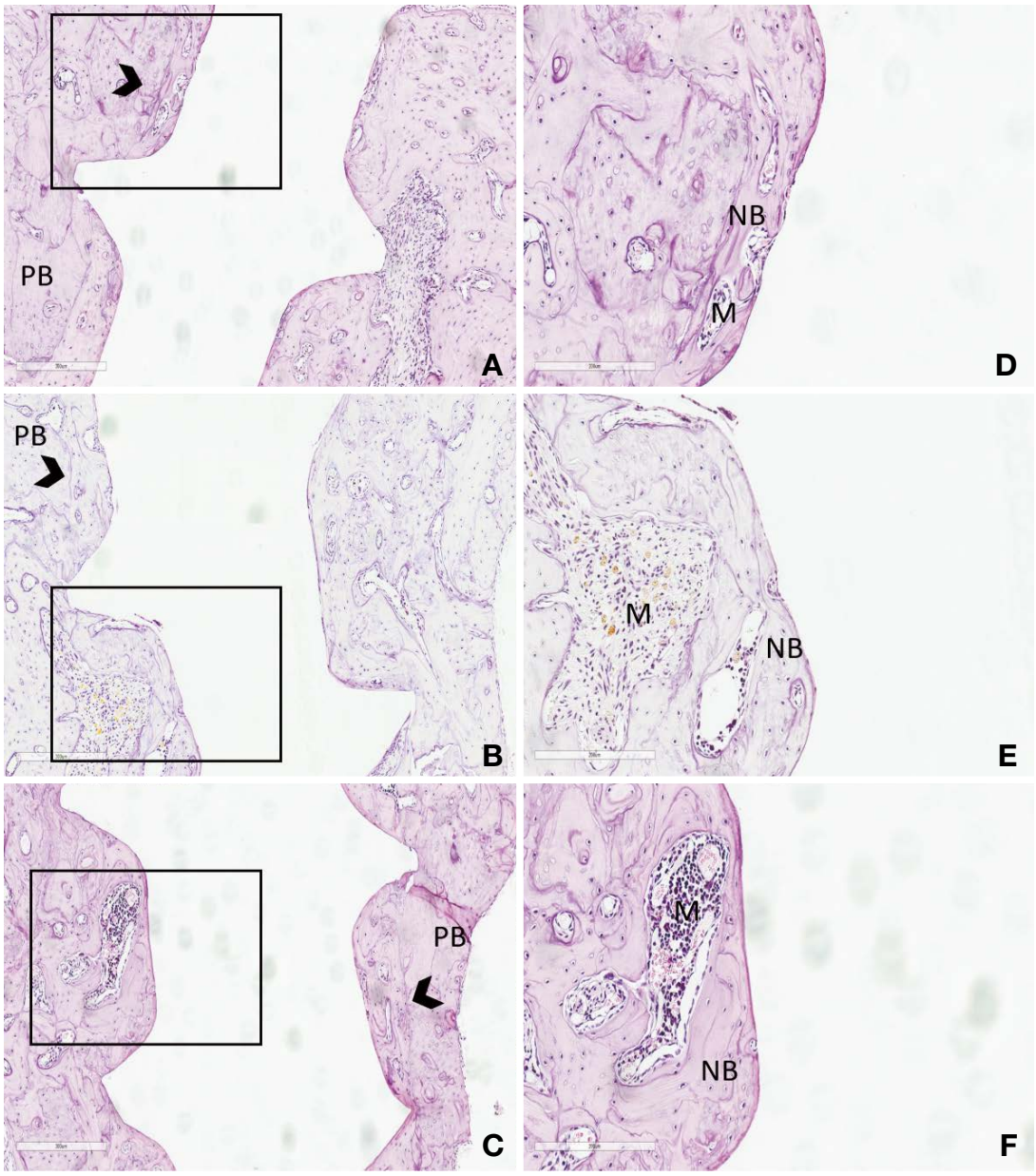

Figure 3. *Representative histologic findings of H & E staining ın the sham-operated control group (A, D), the ovariectomized group (B, E), and the VitD (C, F) at 8 weeks after installation. In the control and VitD groups, complete osseointegration was achieved after implant placement while soft tissue engaged between the implant and bone interface in the OVX group. Arrowhead: cutting line by drill; NB: newly formed bone; PB: previous bone; M: marrow cavity. Left row; 10× magnification, scale bar=200 μm, Right row; 20× magnification, scale bar=100 μm.*

microscope (BE51, Olympus, Tokyo, Japan).

RESULTS

1. Micro-CT findings

The 2D images of the bone around implant in the control and the VitD group showed relatively increased radiologic opacity with relatively well-formed bone. In contrast, the OVX group displayed compromised healing of the bone around the implant with defect in bone filling. In the 3D reconstructed images, the VitD group demonstrated partially covered bone around implants. However, the OVX group

showed relatively thin and spare layer of bone formed around the implant surface (Fig 2).

2. Histologic observations

In some animals of the control and the VitD group, complete osseointegration was achieved at 8 weeks after implant placement. A mass of mature lamellar bone which was calcified to a degree close to that of normal bone tissue in the implant bone interface was observed. Also, there was an extensive area of bone tissue was in direct contact with the implant surface. However, in the OVX group, soft tissue was engaging between the bone tissue and the implant interface. The newly formed lamellar bone tissues showed slightly smaller in thickness than that in the control group (Fig 3).

DISCUSSION

Since vitamin D is a steroid hormone controlling calcium and phosphorus metabolism, deficiency of vitamin D deficiency is a risk factor for osteoporosis and the occurrence of fractures[11,12,18]. Several animal studies suggest that its deficiency of vitamin D negatively affects bone regeneration and the osseointegration of implants might be impaired[15,16,24]. It is suggested that inadequate serum level of vitamin D is also closely associated with bone mineral density (BMI) and affects bone turnover rate[11]. Therefore, deficient vitamin D could induce the secondary hyperparathyroidism to promote the conversion 25(OH)D to 1,25 $(OH)_2D$, thus bone loss will increase by the bone turnover rate in response to the PTH[25].

In contrast, there are few studies that vitamin D supplementation be used in pharmacologic therapy for osteoporosis and osseointegration because vitamin D treatment would support bone formation[20,26-28]. As vitamin D has positive effects on bone metabolism, it is proposed that vitamin D could exert on bone regeneration process[15,24]. The results of this study were similar to the previous studies, and vitamin D administration counteracts the reduced peri-implant bone regeneration caused by ovariectomy. Specifically, vitamin D supplementation has affected on the bone formation and apposition in the bone region around implant threads. In the finding of histologic observation, distinguishable difference in bone formation at 8 weeks after implant installation was not found between vitamin D-administered group and the control group. However, histological appearance of new bone in the OVX group was more woven in nature rather than lamellar. The newly formed trabecula bones in control and vitamin D-administered groups showed mostly similar appearance those were thicker and lamellar structure. Moreover, a large amount of mature lamellated bone around the implant-bone interface was calcified to some degree. Such discrepancy between the control and vitamin D-administered group and OVX group could be associated with vitamin D because it has an anabolic role in proliferation and differentiation of osteoblasts and bone formation[19,26]. Therefore, vitamin D supplementation is an effective treatment for reduced peri-implant bone formation because it directly counteracts the catabolic mechanisms of osteoporosis.

The positive effects of vitamin D administration was the increased peri-implant bone covering pattern of implant surface. As seen in the 3D images of this experiment, peri-implant bones of the control group and the vitamin D-administered were larger and more compact in comparison with those of the OVX group. These findings are consistent with the histologic observation[29].

It has been never studied the impact of vitamin D supplementation on bone formation around implant placed in the maxilla of ovariectomized rats based on histologic and micro CT radiologic observation[16,19,20,27]. The advantage of ovariectomized rat maxilla model used in this study is its resemblance to clinical settings in the field of dentistry. This model was to address the concern of bone healing around titanium implants in the maxilla after implantation in osteoporotic rats. Most of animal studies have performed in the long bones of animals, for example, tibia and femur of osteopenic or osteoporotic animals. However, these studies have the shortcoming that the long bones of animals as sites of implant placement usually showed the favorable transcortical fixation of implants[16,19,20,27]. As far as we know, this study is the first one that investigates bone around implant placed in edentulous alveolar bone of maxilla following tooth extraction. Since the fixation in two thick cortical layers of implant is impossible in the maxilla, the result of present study was meaningful. As shown in representative photographs, the neck of the many implants was not surrounded by the cortical bone. It is required that the careful interpretation of the results.

This study has several limitations. At first, the observation of this experiment had no statistical meanings since it was only representative histological and radiologic findings. Thus, it should be interpreted cautiously for justification of vitamin D administration. Moreover, it is required to examine the statistical significance of increased thickness of peri-implant bone using enough number of animals. Secondly, the osteoporotic rat maxilla model used in this study only showed the

osseointegration process during the healing phase after implant installation. It is needed to be performed in the situation of the oral cavity where implants are continuously subjected to functional loading[28]. Likewise, a long-term functional status of implants also required to be studied in the future. Thirdly, further studies about the mechanism in improving osseointegration of implants by the administration of vitamin D through the molecular biologic research[30]. Finally, it is critical to establish the guidelines for selecting patients, administration timing, and duration of drugs in clinical situation.

CONCLUSION

In summary, findings of this study might support that vitamin D supplement promoted the new bone formation around the implant in osteoporotic rat maxilla. More studies are required to establish the effect of vitamin D treatment on clinical outcome basis.

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