Osteointegration of dental implants is influenced by local and systemic factors. Long-term success and survival rates are greatly affected by the early stability of implants in the stages of osseointegration.

Although dental implant surgery is a widespread treatment option for restoration of oral function, certain patients with systemic health problems are not indicated for implant therapy. 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 is a risk factor for compromised osseointegration.

Osteoporosis is a degenerative bone disease affecting postmenopausal women and individuals older than 50 years of age. Estrogen deficiency after menopause and osteopenia following the aging process cause reduced bone mass and bone quality. This leads to weakened bone strength, and the risks of fractures in the spine, wrist, and hip are considerably increased. According to an epidemiologic study, almost 40% of Korean women older than 50 years of age experience osteoporosis and suffer from the sequelae.

Purpose: Raloxifene, an antiresorptive drug, prevents bone loss and promotes bone formation by secondary anabolic action. The purpose of this study was to investigate the effectiveness of raloxifene on the osseointegration of implants in the rat model of the osteoporotic maxilla.

Materials and Methods: Thirty Sprague-Dawley female rats aged 10 weeks were randomly assigned to the following groups: (1) raloxifene (RAL) group ($n=10$), (2) ovariectomized (OVX) group ($n=10$), and (3) control group (sham-operated, $n=10$). Both ovaries were removed to induce osteoporosis, and the maxillary right molar was extracted. After 4 weeks, an implant was placed on the same edentulous area. Raloxifene 1 mg/kg/day was administered to the experimental animals in the RAL group, while those in the ovariectomized group and sham-operated group were given the same amount. All experimental animals were sacrificed at 4 weeks after implants were placed. Histomorphometric measurements of the bone area ratio (BA, %) and bone-to-implant contact ratio (BIC, %) around the implant were performed. Three-dimensional (3D) microcomputed tomographic (micro-CT) analysis of peri-implant bone microarchitecture was also performed and statistically analyzed.

Results: In the histomorphometric analysis, the BA ratio of the implant in the RAL group was higher than that in the OVX group (53.3% ± 7.2% vs 38.2% ± 11.7%, $P=0.024$). BIC around the implant in the RAL group did not show a statistical difference compared with that in the OVX group (42.7% ± 15.0% vs 34.5% ± 16.2%, $P>0.05$). In microstructural analysis, the RAL group showed a significant increase of trabecular thickness compared with the OVX group (0.09 ± 0.02 mm vs 0.06 ± 0.01 mm, $P=0.013$). However, raloxifene did not show convincing differences between the groups in other micro-CT parameters ($P>0.05$).

Conclusion: The results show that raloxifene administration demonstrated enhanced new peri-implant bone formation in the osteoporotic rat maxilla. Further research is needed to reveal the effect of raloxifene on the clinical outcome of patients with poor bone quality who undergo dental implant treatment.
With an increasing proportion of elderly people due to extended lifespan, the number of age-related osteoporotic individuals is also continuously rising. In addition, half of these osteoporotic patients are unaware of their disease status.7,8 Although clinical evidence is insufficient, osseointegration of dental implants is impaired by an adverse balance of bone turnover and compromised regeneration capacity.5 Therefore, patients with osteoporosis might have a higher risk of losing dental implants than nonosteoporotic patients.7 Since both implant osseointegration and osteoporosis basically have similar bone physiology, osteoporosis treatment can be applied to improve osseointegration of dental implants and bone quality.4,5,8,9 Treatment of osteoporosis includes hormone replacement therapy, bisphosphonates (BPs), calcium supplements, parathyroid hormone, and selective estrogen receptor modulators (SERMs). Among these agents, BPs and SERMs act as antiresorptive drugs that prevent loss of bone mass. It is also known that they exert secondary effects on the processes of bone healing such as fracture and distraction osteogenesis.10–13

SERMs have selective affinity to estrogen receptors of the bone tissue. SERMs prevent bone loss after menopause and decrease the prevalence of vertebrae and hip fracture through reducing the bone turnover and increasing bone mineral density.11,12 It is known that SERMs can inhibit osteoclast differentiation and activity. They can also promote the differentiation and activity of osteoblasts, thus providing positive results in the formation of new bone around the implant in osteoporosis patients.14–17 Raloxifene, one of the SERMs, is approved for treatment of osteoporosis. It stimulates the alveolar bone healing process after tooth extraction and increases the bone mass around implants in osteoporotic rats. Moreover, oral raloxifene can restore the reverse torque and bone-to-implant contact ratio to levels found in the tibia of the nonosteoporotic group.18–21 Thus far, no study has provided sufficient data on the effect of raloxifene on osseointegration using an osteoporotic rat maxilla model. In this study, the authors hypothesized that raloxifene could improve osseointegration of dental implants in the maxilla. To assess the effects of raloxifene treatment, peri-implant bone area (BA) and bone-to-implant contact (BIC) ratio were measured. A microcomputed tomographic (micro-CT) examination was also adopted to recognize changes in trabecular bone architecture.22

**MATERIALS AND METHODS**

**Experimental Animals**

Thirty Sprague-Dawley female rats (mean age: 8 weeks; mean weight: 250 to 300 g) were purchased from Nara Biotech. The room temperature (25°C ± 1°C), humidity (55%), and a daily light condition (12-hour light/dark cycle) were maintained throughout the experiment. The rats were randomly allocated into the following three groups: (1) the raloxifene (RAL) group (n = 10) included ovariectomized rats that were given raloxifene; (2) the ovariectomized (OVX) group (n = 10) included ovariectomized rats only; and (3) the control group (n = 10) included rats that were sham-operated (Fig 1). All surgical and animal handling protocols were approved by the IACUC (BSM 16-001).

To induce the osteoporotic condition, animals in the experimental group underwent bilateral ovariectomy through a dorsal approach, while rats in the control group were sham-operated. Rats in the RAL and ovariectomized groups were provided with a low calcium diet, while rats in the control group were provided a standard rat chow. Water was given ad libitum.

**Surgical Procedures**

The maxillary right first molar was carefully extracted under general anesthesia with a mixture of tiletamine-zolazepam (30 mg/kg, Virbac) and xylazine.
hydroxyapatite (10 mg/kg, Bayer-Korea) 8 weeks after ovariectomy.
Extraction sites were allowed to heal for 4 weeks. Experimental animals were anesthetized again, and a titanium microscrew implant (1.2 × 3 mm in diameter and length) was placed. Using a pilot drill, a hole was prepared on the edentulous area of the maxilla mesial to the second molar. During drilling, copious saline irrigation was used to prevent overheating. Postoperatively, gentamicin (5 mg/kg, Gukje Pharm) and ketoprofen (5 mg/kg, Bugwang Pharm) were given by intramuscular injection for 3 days.

Administration of Raloxifene
Raloxifene hydrochloride 1 mg/kg/day (Sigma-Aldrich) was applied into the stomach using a feeding needle from the day after implant placement for the RAL group. The same amount of saline was administered to the ovariectomized group and the control group.

Preparation of Specimens and Histomorphometric Analysis
All rats were sacrificed by CO₂ intoxication 4 weeks after implant placement. Bone block surrounding the implant was collected and fixed in 10% formalin (Sigma-Aldrich). Samples (n = 5 per group) were dehydrated with ascending grades of ethyl alcohol and embedded in methyl methacrylic resin. After polymerization, specimens were sectioned (50 ± 5 µm in thickness) longitudinal to the implant body. Then, sections were stained with Goldner-trichrome for histomorphometric measurements, which were performed by an experienced examiner blinded to the group assignment of each specimen. Using a microscope (Leica Microsystems) equipped with a digital camera, images were captured. BIC and bone area ratio (BA) were analyzed with i-Solution software (IMT Technology). The BIC ratio was defined as the fraction of directly contacted bone along the surface of the implant within the bone. The BA ratio was defined as the fraction of the bone area within the thread.

Microcomputed Tomography (micro-CT) Analysis
The other specimens (n = 5 per group) were scanned with a micro-CT scanner (Skyscan 1173, Bruker) along the longitudinal axis of the implant in the axial direction. The image pixel size was 5.33 µm and was obtained with a setting at 130 kilovoltage and 45 microampere. Three-dimensional (3D) images were acquired with the Nrecon reconstruction program (Bruker MicroCT). The volume of interest was set as the bone from 1.0 mm below the screw head to 2.5 mm apically extended and 0.3 mm outwardly from the implant perimeter. The ratio of bone volume to total volume (BV/TV, %) was used to describe the quantity of bone. The parameters trabecular thickness (Tb.Th, mm), trabecular number (Tb.N, 1/mm), and trabecular separation (Tb.Sp, mm) were measured to compare the microstructure of the trabecular bone.

Statistical Analysis
To compare values of each parameter according to groups, the nonparametric Kruskal-Wallis test was applied. When significant differences in groups were suggested, a Mann-Whitney test was followed to find significance between groups. The statistical significance level was set at P < .05.

RESULTS
Histomorphometric Assessment
Raised BA ratio and BIC ratio were evident in the rats in the RAL group compared with the rats in the ovariectomized group. At 4 weeks after implantation, the control group showed intimate contact with the bone, and the RAL group also showed an extensive area of direct bony contact with the implant surface. However, soft tissue was intervened between the bone and the implant interface, which explained the compromised bone healing in the ovariectomized group. Representative microscopic findings of each group are shown in Fig 2. Though the BA value of the RAL group did not reach the BA value of the control group, the bone area in the thread region of the RAL group was greater than the ovariectomized group (Table 1). The BA ratio in the RAL group was considerably greater than the BA ratio in the ovariectomized group (P = .024). As expected, the highest value of BIC was observed in the control group. Even though the BIC ratio in the RAL group was greater than the BIC ratio in the ovariectomized group, there was no meaningful difference between the RAL group and the ovariectomized group (P > .05) (Fig 3).

Microcomputed Tomographic Analysis
Micro-CT revealed increased bone volume in the rats in the RAL group, which was enhanced by raloxifene administration. The plain two-dimensional (2D) images of the bone around the implants in the RAL group showed relatively increased radiopacity with partial bone formation. In contrast, the ovariectomized group displayed defective bone healing around the implant with failure in bone filling. In 3D reconstructed images, the RAL group demonstrated partially covered bone around implants. On the other hand, the ovariectomized group hardly showed new bone formed around the implants (Fig 4).

Three-dimensional microstructural parameters of the RAL group showed improved qualities compared
with those of the OVX group in Table 2. At 4 weeks after implantation, the Tb.Th value of the RAL group was considerably greater than the value of the ovariectomized group (P = .013). However, the values of BV/TV, Tb.N, and Tb.Sp of the ovariectomized group were not considerably different from values of the RAL group (P > .05) (Fig 5). Raloxifene treatment had no significant effect on the microstructure of bone around the implant.

**DISCUSSION**

This study tried to confirm the assumption that raloxifene could enhance bone formation and affect the structure of bone around implants in ovariectomy-induced osteoporotic rats. By histomorphometric observation of nondecalcified specimens and micro-CT analysis, the results of the present study partially corroborated with clinical reports describing an encouraging effect of raloxifene treatment on bone formation.11,16,23
In this research, a rat was ovariectomized to produce postmenopausal osteoporosis. Estrogen deficiency caused by ovariectomy can increase bone turnover with substantial bone loss. Some reports revealed lowered bone structural parameters in the maxilla in the ovariectomized model. Though the present study did not evaluate the osteoporotic level by biologic markers, findings from this study are consistent with these previous reports. Osteoporosis induced by ovariectomy mainly affected the bone marrow. As a result, rats in the ovariectomized and RAL groups showed a reduction of the bone volume, number, and thickness of trabecules, but an increase in separation compared with rats in the control group.

Recent studies have demonstrated that raloxifene can enhance peri-implant bone healing in osteoporotic rats and osseointegration of implants by active bone dynamics compared with bisphosphonates. Raloxifene also improved new bone formation around implants in large animals. In accordance with previous studies, the results of this experiment demonstrated that raloxifene exerted an anabolic effect on bone microstructure. As shown in 3D images of this experiment, peri-implant bones of the control group and the RAL group (drawn as yellow halo surrounding the inner screw) were larger and more compact in comparison with those of the ovariectomized group.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>BV (%)</th>
<th>Tb.Th (mm)*</th>
<th>Tb.N (1/mm³)</th>
<th>Tb.Sp (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>42.65 ± 12.7</td>
<td>0.09 ± 0.02</td>
<td>4.85 ± 1.19</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>OVX</td>
<td>5</td>
<td>31.87 ± 4.90</td>
<td>0.06 ± 0.01</td>
<td>3.68 ± 0.46</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>RAL</td>
<td>5</td>
<td>33.27 ± 14.6</td>
<td>0.09 ± 0.02</td>
<td>4.80 ± 1.74</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>.281</td>
<td>.013</td>
<td>.221</td>
<td>.619</td>
</tr>
</tbody>
</table>

All data are expressed as median ± SE. Control = sham-operated control group; OVX = ovariectomized only group; RAL = ovariectomized and raloxifene-administered group; BV = bone volume ratio; Tb.Th = trabecular thickness; Tb.N = trabecular number; Tb.Sp = trabecular separation. *Significantly different between groups by Kruskal-Wallis test.
It is contentious whether raloxifene acts as a preventive tool for bone loss or an enhancer to regenerate bone around implants. Raloxifene is an antiresorptive agent that can decrease bone resorption due to osteoporosis.11,23,29 In one study, SERM enhanced bone formation during bone graft healing by shifting the bone formation-resorption balance positively.30 This could explain why trabecular bone structural parameters in raloxifene-administered rats maintained similar values to those of control rats in the present study.

In this study, the lack of estrogen in the ovariectomized group truly impacted trabecular bone microstructure, resulting in a decrease of parameters of bone architecture. On the contrary, raloxifene enhanced the formation of trabecular bone in raloxifene-administered rats. The result of traditional BIC ratio and BA parameters supported the above assumption. Similar patterns of 3D micro-CT analysis in the control and RAL groups demonstrated that raloxifene could increase bone formation. The result of histomorphometric analysis by BV and BIC was limited in medullary compartments only. Since the cortical compartment has a crucial role in osseointegration of implants, the region of interest needs to be considered.31 Moreover, there were some artifacts by metal effect in micro-CT images. Therefore, implants must be removed to avoid artifacts.32

In histomorphometric analysis, the control and RAL groups generated larger bone areas compared with the ovariectomized group. Additionally, the micro-CT results revealed that the trabecular thickness was the only statistically significant difference between the ovariectomized group and the RAL group. Other parameters did not show considerable differences, although the RAL group tended to have higher numbers of BV and trabecules. Therefore, raloxifene has a tendency to give good results, although the difference is not significant.

In this experiment, raloxifene was administered for 4 weeks after implant placement. Such a relatively short period of administration might have an impact on results. The U.S. Food and Drug Administration (FDA) approved raloxifene at 60 mg/day of oral dosage for preventive and therapeutic purposes for postmenopausal osteoporosis.15,16 However, discontinuation of raloxifene after 5 years of prescription can lead to a decline in bone mineral density in the vertebrae.33

**Fig 5** Boxplot graphs of (a) bone volume, (b) trabecular thickness, (c) trabecular number, and (d) trabecular separation of the peri-implant bone in the rat maxilla at 4 weeks after implantation in the sham-operated control group (Cont), ovariectomized (OVX), and ovariectomized and raloxifene-administered group (RAL). *Significant difference between the control group and the OVX group; **OVX group vs RAL group (P = .016 and P = .028, by Mann-Whitney test, respectively).
Therefore, to maintain a positive effect on bone remodeling, it is recommended to continue raloxifene longer than the period of primary osseointegration. Further study will be needed to decide whether raloxifene can be used following dental implant placement without complications.

Limitations of this study are as follows. First, this study was underpowered due to the small number of animals used. Five animals in one group did not show the normal distribution pattern of data, and large variation in the standard deviations was observed between groups. This prevented the authors from finding statistical significance in the analysis. An appropriate setup of primary endpoint is required in calculation of a sample size that will provide sufficient statistical power. Second, the rat maxilla model does not fully reproduce the clinical circumstance in which implants are functionally loaded during mastication. Therefore, the model used in this study indicates only the early healing phase; it did not reflect the long-term result of functional use of implants in osteoporotic patients. Lastly, this study is rather indecisive because the exact molecular and cellular mechanisms of bone-forming factors with raloxifene remain unknown. In addition, numeric data could not provide the overall impact of raloxifene due to the short period of experimental duration. However, the impact of raloxifene could be obtained from figures, although it is rather inclusive. Nonetheless, this study showed that raloxifene could help bone formation around the implant, at least in osteoporotic rats.

CONCLUSIONS

This study demonstrated that raloxifene administration could enhance peri-implant bone formation in an osteoporotic rat maxillary model. Within the caution of interpreting these results, clinicians could consider the use of raloxifene for osteoporotic patients who undergo dental implant treatment. Further research is suggested to establish the effect of raloxifene on clinical outcomes of patients with poor bone quality.

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REFERENCES