The Influence of Synthetic Bone Mineral to Bone Formation

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Abstract

Dental implant is an effective treatment modality to restore esthetic loss and masticatory dysfunctions. However, at least three to six months healing period following implant surgery is necessary. This healing period is burden for the patients, especially with a broad edentulous area and may decrease their quality of life. Therefore, shortening the healing period and accelerating prosthetic rehabilitation is clinically important. We speculate peri-implant bone may be improved by systematic approaches such as use of osteoporosis supplement promoting bone metabolism. As a preliminary step, an animal pilot study was conducted to confirm whether intake of the synthetic bone mineral (SBM) is effective in bone formation.

To confirm whether intake of a supplement developed for osteoporosis, SBM, is effective in bone formation.

Twelve female Wister rats, 20-week-old were randomly assigned to receive a standardized diet with SBM (n=6) or without SBM (n=6, control).

After 8 weeks, right and left femur were extracted from the rat under CO₂ inhalation and bone mineral density (BMD) and bone flexure strength were determined. Between group differences in bone mechanical strength and BMD at 28 weeks old were analyzed by Mann-Whitney U test.

BMD was significantly higher for the Diet with SBM group at compared to the Diet without SBM group ($P < 0.05$). Pull-out strength was also significantly increased than the Diet without SBM group ($P < 0.05$).

Within the imitations, this study indicates that SBM could be effective in bone formation.

Keywords :

animal study,
supplement, Synthetic bone mineral,
bone formation

Introduction

Several studies have indicated that due to an increase in life expectancy, the total number of edentulous patients will increase over the next two decades(1, 2). This implies an increase in the number of edentate patients requiring prosthetic rehabilitation, either by conventional or implant. Based on several randomized and non-randomized controlled trials, mandibular implant overdentures retained by two-ball attachments have been shown considerably effective

in the treatment of edentulism(3, 4).

The implant treatment is an effective modality as mentioned; however, even though patients want to recover esthetic and chewing ability with prostheses as soon as possible, they must wait for several months to obtain an osseointegration between implant and peri-implant bone(5). The healing period is burden for edentulous individuals since this period may decreases masticatory function and patients' quality of life. One investigation reported that 80% of patients who received implant treatment respond that the treatment period was too long(6). Therefore it is important to shorten the healing period and reconstruction the oral

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function by final prosthesis.

There are several reports to shorten healing period including surgical procedure, implant design, surface topography, and surface chemistry(7). With surgical methods, technique of undersized drilling or bone condensing prior to implant placement are recommended to increase bone density in peri-implant region. Recently, new approaches have been arisen by improving the interface between implant and peri-implant bone tissue. Implant materials and/or implant surface treatments have been developed to augment cell adhesion phenomena and protein adsorption at the implant-bone tissue interface. Ogawa(8) reported that titanium surfaces treated with ultraviolet light adopt a unique electrostatic status and act as direct cell attractants to effectively reduce the osseointegration period without the aid of ionic or organic bridges, which imparts a novel physicochemical functionality to titanium(9). Vibratory stimulation has also been reported to improve bone-to-implant contact ratio(10). These research focused to improve osseointegration between the implant body and locally surrounded bone tissue.

In general medicine, systematic approach such as supplemental therapy for osteoporosis to improve bone metabolism has been investigated(11, 12). LeGeros developed a calcium phosphate-based supplement incorporating magnesium (Mg), zinc (Zn), fluoride (F) and carbonate, which is called synthetic bone mineral (SBM), to promote bone formation and inhibit bone resorption for osteoporosis(13). The Mg deficiency results in bone loss(14). The Zn reduced the expression level of mRNA in cathepsin and carbonic anhydrase, resulting in inhibition of the development of osteoclast(15). The F promotes the differentiation of osteoblasts by increasing the total collagen content and ALP activity(16, 17). We considered application of SBM to shorten healing period and to improve implant fixation.

As a preliminary step, we conducted an animal pilot study to confirm whether intake of the SBM is effective in bone formation. The null hypothesis of the current study was there is no significant different of bone mineral density (BMD) and bone flexure strength between rats fed by diet without SBM and by diet with SBM.

Materials and Methods

Animal diet

AIN-93M, developed by the American Institute of Nutrition Committee and prepared by Nosan Co.

Table 1 Mineral compositions(Wt%)of diets without and with SBM

	Diet without SBM	Diet with SBM
Calcium (Ca)	0.51	0.74
Phosphate (P)	0.30	0.48
Magnesium (Mg)	0.05	0.35
Zinc (Zn)	0.003	0.036
Fluorine (F)	0	0.005
Carbonate (CO ₃)	0	0.12
Sodium (Na)	0.10	0.13
Potassium (K)	0.35	0.75
Chlorine (Cl)	0.16	0.17

SBM: synthetic bone mineral

(Yokohama, Japan) was used as the control diet, Diet without SBM. The experimental diet was composition of AIN-93M and SBM, Diet with SBM. The SBM was prepared according to LeGeros' recipe(13). Briefly, a mixture of dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) and magnesium and zinc chlorides (MgCl_2 and ZnCl_2 , respectively) were hydrolyzed in double-distilled water containing dissolved potassium carbonate and sodium fluoride. Then the SBM was added to AIN-93M, the mineral composition of which was adjusted by Mijares' method (18). The composition of the diets without and with SBM is shown in Table 1.

Animal experiment

Twelve female Wister rats, 20-week-old (Sankyo Labo Service, Tokyo, Japan) were used. After consuming a diet without SBM for 1 week to acclimate to changes in their environment, 20-week-old rats were randomly allocated to one of two groups: a control group fed without SBM (n=6) or an experimental group fed with SBM (n=6). From each group, 6 rats were used for bone flexure strength measurement and BMD analyses. Rats were housed individually with food and water ad libitum, with temperature and relative humidity maintained at $25 \pm 1^\circ\text{C}$ and $50 \pm 1\%$, respectively. After 8 weeks, the rats were sacrificed by CO_2 inhalation by utilizing a CO_2 chamber. The study protocol was approved by the Ethical Committee of Nihon University (AP12-MD017).

Bone flexure strength

The right femur was extracted and soft tissue was

Fig.1 Bone flexure strength

The femur bone was supported using a 3-point bending jig with a between-fulcrum distance of 10 mm, and a bending force was loaded on the middle point of the bone length at 5.0 mm/min.

removed by gauze. Bone mechanical of strength was evaluated by flexure strength. The right femur was placed in physiological saline and stored at 8°C for 24 h. The bone was dried with a sterile cloth at the time of the test. Bone flexure strength was measured using an Instron type testing machine system (TG-5k, Minebea Co., Kanagawa, Japan). The femur bone was supported using a 3-point bending jig with a between-fulcrum distance of 10 mm (Fig. 1), and a bending force was loaded on the middle point (2.0 mm) of the bone length at 5.0 mm/min. The median value was calculated from the data of bone flexure strength.

BMD and BMD color imaging

Micro computed tomography imaging of the left femur was taken by the R_mCT (Rigaku, Tokyo, Japan) operating with an anode electrical current of 90 kV at resolution of 30 μ m. The isotropic resolution was 30 $\times$ 30 $\times$ 30 μ m voxels. Analyzed regions were selected by setting the measurement range at the mid-point of the full femur length (Fig. 2). The R_mCT Image Analysis software (Rigaku, Tokyo, Japan) generated 3-dimensional models using scan data. The TRI/3D-BON image analyzer (Ratoc System Engineering, Tokyo, Japan) was used to calculate BMD of the femur mid-shaft bone and generate color images depicting BMD intensity, with blue and light blue, green and yellow, and orange and red representing low, medium, and high BMD, respectively.

Statistical analysis

Between group differences in bone mechanical strength

Fig.2 Scanning of femur mid-shaft for BMD analyses

Analysis sites in the femur (top). The measurement range (4.0 mm $\times$ 4.0 mm $\times$ 2.0 mm) for BMD is at the femoral mid-shaft.

and BMD were analyzed by Mann-Whitney U test. All statistical analyses were performed using the statistical package PASW[®] Statistics 18.0 (SPSS, IL, USA). A *P* value below 0.05 was considered statistically significant.

Results

Bone flexure strength (Fig. 3A)

The bone strength in the Diet without SBM and Diet with SBM groups was 164.2 MPa and 177.8 MPa, respectively of median value. Mann-Whitney U test showed that the bone strength was significantly higher than those of Diet without SBM groups (*P* < 0.05).

BMD (Fig. 3B)

The BMD in the femoral mid-shaft cortical bone of Diet without SBM group and Diet with SBM group was 1261.9 mg/cm³ and 1295.6 mg/cm³ respectively of median value. Mann-Whitney U test showed that the BMD in the femoral mid-shaft cortical bone was significantly greater than those of Diet without SBM groups (*P* < 0.05).

BMD color imaging (Fig. 4)

The Diet without SBM group observed mainly with orange color (Fig. 4A). The Diet with SBM group observed with orange and red spectrum (Fig. 4B). However, the Diet with SBM group mainly occupied the orange and red end of the spectrum.

Fig.3 Between group differences in bone mechanical strength and BMD.

Results of bone mechanical strength (A): Bone flexure strength was significantly higher than those of Diet without SBM groups ($P < 0.05$).

Results of BMD (B): BMD was significantly higher than those of Diet without SBM groups ($P < 0.05$).

Fig.4 BMD color image and polarized microscopic images

BMD color image observation: Color imaging of the Diet without SBM group revealed mainly orange (A). In contrast, the color imaging of the Diet with SBM group occupied mainly the orange and red end of the spectrum (B).

Discussion

This animal study revealed that rats fed with SBM increased flexural strength and BMD of the femur bone quantitatively than rats fed without SBM, indicating the intake of the SBM was effective in bone formation. Additionally, the increased BMD observed qualitatively, by BMD color images in rats fed by SBM at 28 weeks, showing majority of areas dominated increase of bone formation. This result agreed with the previous report that revealed correlation between flexural strength of bone related and increase of BMD (19).

The difference of intervention to two groups is only the difference fed either with or without SBM. Therefore difference of BMD mineral composition, 7 times of Mg and

12 times of Zn and F for Diet with SBM, may affect mainly with bone formation. The Mg, Zn and F play important roles of bone formation and resorption. The Mg deficiency results in bone loss (14). The Zn reduced the expression level of mRNA in cathepsin and carbonic anhydrase, resulting in inhibition of the development of osteoclast (15). The F promotes the differentiation of osteoblasts by increasing the total collagen content and ALP activity (16, 17). Mijares et al (18) stated that the effect of SBM may be explained in terms of individual and combined effects of Mg, Zn and F on bone cell activities such as bone formation and bone resorption when released from the SBM or when incorporated in newly formed bone. These reports might explain why the rats fed by diet with SBM had greater BMD than

those without SBM.

In the future, elderly people would select implant treatment reflecting an aging society.

The SBM was originally developed for the prevention and treatment of osteoporosis in preparation for the aging of society. Therefore, from the osteoporosis and implant treatment in aging, the SBM supplement is fascinate to study furthermore. As a preliminarily step, in this study concluded that the SBM could be effective in bone formation.

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