The international population report estimated that the percentage of population aged 65 and older in the world would double by the year 2050.\textsuperscript{1} Due to severe alveolar ridge resorption and systemic medical conditions (such as diabetes, osteoporosis), elderly patients face many challenges in implant prognosis. Though dental research has paid considerable attention to the effect of age on implant prognosis, the underlying mechanism remains controversial. It was reported that advanced age was a potential contraindication to the success of osseointegration.\textsuperscript{2,3} One retrospective study involving 1,140 patients claimed that patients aged 60 and older experienced a twofold higher risk of implant failure compared with patients less than 40 years of age.\textsuperscript{4} However, there is also scientific evidence from reviews and meta-analyses reporting that age alone should not be a major prognostic factor in implant treatment, whereas bone quantity and bone quality or implant features might be more critical to a favorable result.\textsuperscript{5–7}

In recent years, it has been commonly accepted that many factors, including biomechanical condition and host bone quality and quantity, play essential roles in implant success.\textsuperscript{8} The rigid connection between the implant and bone lacks force-absorbing capability; as a result, the implant is much more sensitive to occlusal overloading compared with the natural tooth. Based

### Purpose: Biomechanical Role of Trabecular Microstructure on Peri-implant Strain Based on Micro-CT Finite Element Modeling of Aging Mice

**Purpose:** To evaluate the influence of age and trabecular microstructure on peri-implant strain in aging and young mice models under compressive load. **Materials and Methods:** Eighteen 4-week-old female C57BL/6 mice (n = 6) were subjected to a 1.2% calcium content diet (young normal calcium group), and 7-month-old mice (n = 12) were randomly subjected to 0.01% and 1.2% calcium content diets (aging low and normal calcium groups, respectively) for 3 weeks. Histomorphometric and microcomputed tomography (micro-CT) analyses were used to investigate local alveolar bone microstructure. One maxilla segment from each group was reconstructed using micro-CT images to highlight the trabecular microstructure. A finite element analysis based on a computational model of the maxilla segment was performed to investigate peri-implant strain. Implants with three different diameters (0.3, 0.4, and 0.5 mm) were analyzed in these models. **Results:** The aging low calcium group showed worse cancellous microstructure in hematoxylin and eosin (HE) staining, significantly increased osteoclast numbers ($P < .05$), and reduced bone volume fraction and trabecular thickness compared with the aging normal calcium group ($P < .05$). However, the young normal calcium group presented no difference in trabecular microstructure and osteoclast numbers compared with the aging normal calcium group. The aging low calcium group demonstrated increased strain intensity compared with the aging normal calcium group, whereas the young normal calcium and aging normal calcium groups showed comparable strain magnitude. The strain intensity of peri-implant bone increased with worse cancellous microstructure. When the diameter increased from 0.3 mm to 0.4 mm, the percentages of pathologic overload decreased regardless of bone microstructure. **Conclusion:** Deteriorated bone microstructure induced by a low calcium diet determined higher strain intensity, whereas, whenever age had no significant effect on trabecular microstructure, consequently, there was no substantial influence on strain. An increase of implant diameters can improve the strain distribution. Clinical decision-making should take into consideration the patient-specific and site-specific trabecular microstructure in preoperative assessment.

**Keywords:** aging, dental implants, finite element analysis, strains, x-ray microtomography

on Wolff’s Law, strain distribution is one of the decisive criteria in the formation and maintenance of implant osseointegration. The naturally "permissible" strains, 100 to 2,000 microstrain, can maintain implant stability. If the bone strain surpasses 1,500 microstrain repeatedly, it can cause little microscopic fatigue damage (MDx), which can be repaired by load-bearing bone. However, when the strain surpasses 3,000 microstrain, the MDx may escape repair and accumulate, leading to damage to the original bone modeling/remodeling equilibrium, finally resulting in bone resorption and implant loosening. Thus, understanding the strain distribution of peri-implant bone is a key factor to predict long-term prognosis of implant treatment.

In the last four decades, finite element analysis (FEA) has become an indispensable technique to investigate the biomechanical bone response during load transfer. Previous FEA studies claimed that lower bone density could significantly increase cancellous bone strain/stress. However, in the early finite element simulations, the cancellous bone was commonly simplified as a continuous bone block. It is widely known that bone quality is influenced by the internal structure of bone. Moreover, mechanical properties of bone depend not only on elastic modulus, but also on the density and organization of the trabeculae. With the improved resolution of micro-CT and the development of the FEA system, it is now possible to reconstruct the cancellous bone precisely and gain further understanding of the biomechanical role of the trabecular microstructure. It has been reported that the actual complex trabecular microstructure is crucial to biomechanical analysis in bone since it significantly affected the mechanical stimulus intensity and distribution pattern. Also, it has been observed that the refined model with an actual trabecular structure presented a broader distribution pattern and decreased strain intensity compared with the continuous models. However, these previous studies did not engage with how the deteriorated bone microstructure with poor bone quality, which can be assumed especially for an aging population, affects peri-implant stress/strain.

Therefore, to better understand the implant performances in elderly patients, the present study aimed to use the micro-CT-based FEA method to investigate the biomechanical risk factors that affect the implant prognosis in the aging population. Special attention was paid to the influence of trabecular microstructure and implant diameter on peri-implant strain distribution.

**Materials and Methods**

**Animal Model**

Female young (4-week, n = 6) vs aging (approximately 7-month, n = 12) C57BL/6 mice were used in the experiment. Young mice were subjected to a 1.2% calcium content diet (young normal calcium group) for 3 weeks. Aging mice were randomly divided into two groups, then subjected to 0.01% and 1.2% calcium content diets (aging low and normal calcium groups, respectively) for 3 weeks. The mice were sacrificed with an overdose of anesthetic prior to cervical dislocation, and maxillae were dissected for experiments. The animal study was conducted with the approval of IACUC of Loma Linda University.

**Bone Histomorphometric Analysis**

Bone histomorphometric analysis was used to detect bone structure and osteoclasts in alveolar bones. Half of the maxillae were fixed with 4% paraformaldehyde, decalcified, dehydrated, embedded in paraffin, and sectioned on a 7-μm thickness. The coronal sections were prepared using Microtome (Leica RM2125, Leica). The cutting angle was approximately parallel to the longitudinal axis of molars. Hematoxylin and eosin (HE) staining and tartrate-resistant acid phosphate (TRAP) staining were performed.

**Bone Micro-CT Analysis**

The cancellous bone microstructure was evaluated with micro-CT analysis. Half of the maxillae were dissected, fixed in 70% ethanol, and stored at 4°C for scanning using a micro-CT system (Skyscan 1272, Bruker) with 4.000036-μm voxel size. The trabecular morphometry was measured for bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular spacing separation (Tb.Sp) by CTAn software (Bruker). Trabecular morphometry was quantified within a region of interest (ROI, 0.86 mm × 0.74 mm × 0.92 mm in x/y/z-direction) located at the palatal bone between the second and third molar (Fig 1). The measurement terminology and units used for micro-CT analysis were those recommended by Bouxsein et al.

**Finite Element Analysis**

One micro-CT volume for each group was imported into Simpleware ScanIP (Synopsys) to perform a segmentation of the ROI. Then, 3D models of maxillae, including the trabecular microstructure, were reconstructed based on grayscale value. Three cylindrical and threaded implant models were designed using Ansys Workbench 19.0 (ANSYS) software and were virtually positioned into maxillae models. Implant diameters were set as 0.3, 0.4, and 0.5 mm with a constant 0.4-mm implant length. Subsequently, Simpleware ScanIP was used to perform a Boolean operation to subtract the implant from the bone for creating an implant socket. The resulting implant-bone complex was independently discretized with solid elements of 0.05-mm element size (quadratic, straight-edge tetrahedrons). The
biomechanical volume of interest (VOI), a hollow cylinder with a 0.1-mm-thickness bone wall close to the implant-bone interface and with an axis coincident with the long axis of the implant, was refined with 0.025-mm element size (Fig 1). The mesh model size ranged from 276,732 to 490,073 elements and 486,512 to 855,915 nodes for the bones, and from 111,648 to 162,637 elements and 186,440 to 270,680 nodes for the implants.

Maxillae and dental implants were modeled as homogenous, isotropic, and linearly elastic materials, described by Young modulus (E) and Poisson ratio (μ). The material characteristic of cortical and trabecular bone was assumed to be similar at the micro-level in keeping with the literature. Therefore, the same material properties were used for both bone tissues, specifically E = 14.4 GPa and μ = 0.3. Respectively, those used for the implants were E = 110 GPa and μ = 0.35. The bone-implant interface was defined as a continuous bond assuming a perfect osseointegration and no mutual movement between the two components. Nodes at the mesial and distal extremities were fixed in all directions to represent continuity within the maxilla. A compressive load of 1.24 N (parallel to the long axis of the implant) was applied to the uppermost cross section of the implant. In a previous study, 150-N occlusal force was applied on a 3.3-mm-diameter implant. The load of 1.24 N was selected to generate a similar load level per unit square on the uppermost cross section of the implant. Final results were recorded as equivalent elastic strain (in microstrain, με) in addition to contour plots of equivalent elastic strain.

**RESULTS**

**Bone Histomorphometric Analysis**

Bone histomorphometric analysis revealed that a low calcium diet resulted in worse cancellous microstructure and significantly increased osteoclast number compared with a normal calcium diet in the aging groups (P < .05), whereas no significant effects were demonstrated in trabecular structure and osteoclast number between the young and aging groups with a normal calcium diet. In the aging groups, HE staining showed reduced bone volume and increased cancellous porosity with a low calcium diet compared with a normal calcium diet (Fig 2a). This effect was consistent with thinner trabecular thickness analyzed by micro-CT. In the normal calcium diet groups, young mice presented similar bone volume and cancellous porosity compared with aging mice. Osteoclast was defined as a wine red-stained cell with multi-nuclei, which should be located at the surface of trabecula after TRAP staining (Fig 2c). In the aging groups, the TRAP staining demonstrated significantly (P < .05) increased osteoclast number (31.1 ± 2.55) compared with the normal calcium group (5.88 ± 1.04). In the normal calcium groups, osteoclast number did not show a significant difference between the young group (7.63 ± 0.84) and the aging group (5.88 ± 1.04).

**Bone Micro-CT Analysis**

The bone micro-CT analysis showed that a low calcium diet induced lower bone volume and thinner trabeculae compared with a normal calcium diet in the aging groups, whereas there was no significant difference in bone microstructure between the young and aging groups with the same normal calcium diet. In the aging groups, bone volume fraction (66.42 ± 0.74) and trabecular thickness (0.07 ± 0.00) were significantly (P < .05) reduced with the low calcium diet compared
Fig 2  Bone histomorphometric analysis in young normal calcium (YN), aging normal calcium (AN), and aging low calcium (AL) groups. The low calcium diet induced poor cancellous microstructure and significantly increased osteoclast number ($P < .05$) compared with the high calcium diet in aging groups, whereas age alone showed no significant effect on trabecular microstructure and osteoclast number. (a, b) HE staining of alveolar bones. (c) TRAP staining of alveolar bones. (d) Comparison of osteoclast number between aging low calcium and aging normal calcium/aging normal calcium and young normal calcium groups. *$P < .05$. 
with the normal calcium diet (71.53 ± 1.05, 0.09 ± 0.00); however, trabecular number and trabecular separation presented no significant difference between the low calcium diet (8.95 ± 0.35 and 0.07 ± 0.00) and the normal calcium diet (8.30 ± 0.02 and 0.06 ± 0.00). In the normal calcium groups, bone volume fraction, trabecular thickness, trabecular number, and trabecular separation demonstrated no significant difference between young mice (69.25 ± 3.47, 0.08 ± 0.00, 8.50 ± 0.20, and 0.06 ± 0.00, respectively) and aging mice (71.53 ± 1.05, 0.09 ± 0.00, 8.30 ± 0.02, and 0.06 ± 0.00, respectively; Fig 3).

**Finite Element Analysis**

The strain intensities were subdivided into five intervals corresponding to strain thresholds of the Mechanostat hypothesis: (min, 100 με), (100 με – 1,000 με), (1,000 με – 1,500 με), (1,500 με – 3,000 με), and (3,000 με – max). Strain intensity > 3,000 με can represent pathologic overload, resulting in bone microdamage in implant surrounding bone.9

Under 1.24-N compressive load, the strain was evenly distributed toward a deep and broad region in cancellous bone (Fig 4). In the aging groups, the mice with a low calcium diet presented a greater high strain concentration, which was located at the bone surface and...
apical two-thirds of surrounding bone compared with a normal calcium diet. In the normal calcium groups, young mice showed similar strain distribution patterns with aging mice.

For the quantitative assessment, the percentages of finite elements associated with five intervals were counted and presented in Fig 5. With 0.3-mm-diameter implants, the percentages of pathologic overload were 0.07%, 0.01%, and 0.01% in the aging low calcium, aging normal calcium, and young normal calcium groups, respectively. With 0.4-mm- and 0.5-mm-diameter implants, sharing the same level values, the percentages were 0.02%, 0.00%, and 0.00% in the aging low calcium, aging normal calcium, and young normal calcium groups, respectively.

When the implant diameter increased from 0.3 mm to 0.4 mm, the percentages of pathologic overload decreased 74.43%, 100%, and 100% in the aging low calcium, aging normal calcium, and young normal calcium groups, respectively. However, when the diameter increased from 0.4 mm to 0.5 mm, the percentages of pathologic overload were negligible and showed low differences.

**DISCUSSION**

The present study aimed to investigate whether the biomechanical risk factors of unfavorable implant performance are related to age. Deteriorated bone
Bone quality is believed to be a determinant of long-term implant success; similarly, aging is considered to be a potent risk factor for long-term implant success. Insufficient bone quantity and systemic osteoporosis that are commonly related to elderly patients may be relative contraindications for dental implant therapy. The micro-CT analysis and histomorphometric analysis of the sites of implantation (palatal bone between the second and third molar) showed that a low calcium diet resulted in worse cancellous microstructure in aging mice groups, while the trabecular morphometry showed no difference between aging and young mice with the normal calcium diet. As for C57BL/6 mice, cancellous bone volume decreased from 6 weeks to 24 months of age. In this study, 7-month-old mice did not show significant changes in cancellous bone structure due to the time limit. Meanwhile, the greatest clinical failure was reported for the maxillary posterior region. Generally, bone types III and IV were observed in the maxilla. Mainly, type IV bone was seen in the molar region. As can be seen from the micro-CT images (Fig 3), the palatal bone in the maxillae showed similar internal bone structure to the type IV bone, a large number of cancellous bone surrounded by thin cortical bone.

Understanding the strain distribution of peri-implant bone is a decisive factor to predict the prognosis of implant treatment. The biomechanical behavior of the bone-to-implant contact area (BIC) depends on the type of occlusal loading, implant configuration, the design of the prosthesis, and the quality and quantity of peri-implant bone. The 3D FEA is the widely used and most convenient method to evaluate these parameters. These numerical results can be helpful in a preclinical experiment, but since FEA is a numerical method that just represents simplification of the investigated objects, the results only allow approximation of strain distribution and overloading risk of BIC and statistical analysis is not recommended. In real clinical scenarios, the superstructure has various prosthesis types, materials, and complex connection interfaces, which all play an important role in load transfer. The aim of this study was to investigate the influence of trabecular microstructure on peri-implant strain. Thus, the present study focused on the internal structure of bone and simplified the type of loading and prosthesis. The occlusal loading was simplified as a vertical load on the uppermost cross section of the implant without any superstructure. The present study chose placement of implants virtually in the preclinical trial. Although the presence of cancellous bone porosity replicates the real state of BIC, the virtual implantation just simulates the immediate situation after implantation. When osseointegration is completed, the trabecular microstructure will be aligned in accordance with the load after occlusal loading. The influence of trabecular microstructure on peri-implant strain in a real scenario needs to be confirmed in further in vivo experiments.

The present study reconstructed the hyperfine internal structure of maxillae. Although maxillae were assumed as homogenous, isotropic, and linearly elastic materials, the explicit advantage of modeling the trabecular microstructure of maxillae is that anisotropy is naturally accounted for by means of structural properties. The direction-dependent, anisotropic behavior of the bone tissue is automatically taken into account. Another advantage of the refined 3D FE model is that the load-transfer mechanisms can be represented in much more detail. The cancellous microstructure is an indispensable factor for a bone biomechanical study, as it is of great importance in distributing strain and serving as load buffers. The strain assessment at the trabecular microstructure level with FEA can precisely simulate a real biomechanical environment of the implant-bone complex. As the cross section of an individual trabecula is one of the critical criteria for biomechanical behavior of cancellous bone, and trabecular thickness presents high inter-specimen heterogeneity, the peak strain cannot represent the overall difference of strain state between specific sites. Thus, in the present study, VOIs were created, representing the direct influence zone of implants; then, the percentages of finite elements associated with the Mechanostat-based strain interval within VOIs were assessed. The FEA results from this study showed that the strain was evenly distributed toward a deep and broad region in cancellous bone. The aging mice with a low calcium diet presented much higher strain concentration and higher bone strain intensity, whereas the young and old mice with a normal calcium diet demonstrated comparable strain distribution patterns. This is consistent with previous findings that a less dense trabecular microstructure led to an increased strain in the region of alveolar bone, and an increase of peri-implant bone loss was observed in the osteoporosis group. Therefore, this indicated that deteriorated bone microstructure has a significant negative effect on peri-implant strain, but age alone seems to have no substantial influence on the strain distribution within the age range of this study.

The use of large-diameter implants is recommended in the implantation sites with poor bone quality in order to ensure treatment predictability. Several studies determined that larger-diameter implants could reduce maximum strain and improve the strain distribution pattern. Li et al reported that cancellous bone stress decreased by 27% when implant diameter...
increased from 3.0 to 5.0 mm under axial load in type IV bone. The result in this study showed that the 0.4-mm-diameter implants reduced bone strain regardless of cancellous microstructure compared with 0.3 mm, which was in good agreement with clinical treatment recommendation and previous studies. This could be explained by the large-diameter implants increasing the BIC surface.

Further in vivo and clinical studies are needed to demonstrate the influence of trabecular microstructure on peri-implant strain distribution after bone remodeling under occlusal loading in certain clinical scenarios.

CONCLUSIONS

Based on the results and within the age range of this study, the following can be concluded:

- Low calcium diet–induced bone deterioration in aging mice markedly impacts trabecular microstructure. However, age itself has no significant effect on trabecular microstructure with a normal calcium diet.
- Deteriorated bone structure determines higher bone strain intensity in alveolar bone, whereas, whenever age has no significant effect on trabecular microstructure, consequently, there is no substantial influence on peri-implant strain.
- The use of a large-diameter implant can reduce pathologic overload and improve the strain distribution pattern.

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REFERENCES