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Decoupled effects of bone mass, microarchitecture and tissue property on the mechanical deterioration of osteoporotic bones

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ABSTRACT

Based on the theory of composite mechanics, a three-pillar framework "bone mass-microarchitecture-tissue property" instead of "bone mass-bone quality", is proposed to quantitively characterize the mechanical deterioration of osteoporotic cancellous bones related to the three aspects, and accordingly the individual and integrative influences of bone mass, microarchitecture and tissue property on the mechanical properties of cancellous bones are investigated via the μCT-based finite element method (FEM) simulations of bone samples from healthy and ovariectomy-induced osteoporosis mice. Comparisons among the healthy, mild osteoporotic and severe osteoporotic bones clearly show that the healthy bones have a larger bone volume density and an optimized architecture exhibiting longitudinal superiority able to more efficiently resist the daily loadings which are commonly along their longitudinal direction, while osteoporosis does not only significantly reduce the bone volume density but also greatly deteriorate the microarchitectural topology, resulting in a distinct reduction in the magnitude of effective Young's moduli and a breakdown of the longitudinal superiority as well. Furthermore, through modeling in silico we decoupled the three major factors to probe into their individual effects on the mechanical deterioration of osteoporotic bones. It was found that sole bone loss would exponentially decrease the effective Young's moduli of cancellous bones, microarchitecture plays a dominant role in defining the anisotropic characteristics of bones such as the longitudinal superiority; and change of tissue property seems having a slight and linear influence on the effective Young's moduli of cancellous bones.

1. Introduction

According to the National Institute of Health Consensus Development Conference Statement [1], osteoporosis (OP) was defined as a skeletal disease characterized by low bone strength and high risk of bone fracture. There are around 40% of white postmenopausal female affected by OP (mainly Type I), and the lifetime fracture risk of an OP patient is as high as 40% [2]. Cancellous bone (also known as trabecular bone) well known as porous bone tissue has tremendous surface area where osteoblast and osteoclast work more actively, and it is more susceptible to OP and usually suffers more deterioration in strength once OP occurs. On the other hand, cancellous bone plays a critical role in load transfer and energy absorption in major joints, for instance spine, hip and wrist. Therefore, osteoporotic fractures usually occur at the trabecula-dominant bone sites such as spine, hip or wrist, and probably result in the loss of mobility and autonomy indicating a major drop in quality of life, especially for the aged patients, and also cause a major financial burden on health-care systems over the world [3]. As the worldwide population is continuing aging, the yearly number of fractures and the yearly expenditure are likely to rise substantially in the future. Thus, OP has long been a serious public health issue worldwide [4].

To date, there still lacks an accurate measure of overall bone strength. Bone mineral density (BMD), a measure of bone mass, is widely utilized for OP diagnosis and treatment efficacy evaluation in clinics [5], even though the misperception that OP is equal to bone loss has been well acknowledged [1]. BMD is usually accessed by dual energy X-ray absorptiometry (DXA), representing the total mineral density in a selected bone volume. However, the BMD only can approximately account for 70% of bone strength [1], and the limitation of BMD cannot be negligible because it only reflects the overall bone quantity (mass) to some extent and provides no information on bone microarchitecture, remodeling rate, intrinsic tissue material properties, and so forth [6–8]. In particular, fair and even negative correlation between the increase of

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BMD and decrease of bone fracture risk was frequently reported in the literature [9,10]. In addition, the past several decades have witnessed great advances of stereology making it more convenient to obtain the three-dimensional morphology of bone [11–14]. The bone volume fraction (BV/TV) derived from the three-dimensional images is supposed to be a more direct measure of bone mass and bone loss [11].

After realizing the limitation of BMD, bone strength is suggested to be determined by the integration of two major aspects: bone mass and bone quality. Bone mass can be measured by bone density (i.e., grams of mineral per area or volume) or bone volume ratio (i.e., volume of bone tissue over total volume, BV/TV) as mentioned above. However, bone quality here is just an ambiguous concept relative to bone mass, covering all the bone features that cannot be explained by bone mass (or BMD), ranging from architectures, mineralization, remodeling rate, to damage accumulation, etc [7]. Consequently, bone quality is difficult to be quantitatively characterized by a single measure. From perspective of composite mechanics, cancellous bone can be regarded as a two-phase composite, with one phase being the bone tissue and the other as porosity. Thus, according to the composite theory, three major aspects: bone volume fraction (BV/TV), microarchitecture, and bone tissue properties as shown in Fig. 1, can be figured out to define the mechanical properties of cancellous bone [7,10,15]. The first aspect is directly related to bone mass, while the second and third aspect represent the three-dimensional spatial distribution and mechanical properties of bone material, respectively. The latter two terms together can well describe the bone quality from the point of view of composite mechanics. The three-pillar framework is expected to facilitate the quantitative studies on the roles of bone mass and quality in defining the overall bone strength. However, the separate and integrative effects of the three aspects on bone mechanical properties are still not well understood [8,10,16]. The current paper will systematically investigate the roles of bone volume ratio, microarchitecture and bone tissue properties on the mechanical deterioration of osteoporotic bone, and aim to gain some novel insights in diagnosis, assessment and treatment of OP.

Many researches have clearly shown that the microarchitecture of cancellous bone has great influence on its mechanical behaviors [8,17]. Benefiting from high resolution micro-computed tomography (μCT), the topological analysis of cancellous bone has become clearer than ever. Apart from BV/TV, more detailed information including thickness of trabeculae (Tb.Th), separation of trabeculae (Tb.Sp), trabecula number (Tb.N), structural model index (SMI) and connectivity can be obtained [18,19]. 3D digital topological analyses in-vivo and in-vitro showed that there are distinct differences between healthy and OP bones in microarchitecture [12,13,20]. For instance, through stereological analysis of μCT data and mechanical testing Ciarelli et al. comparatively studied the architecture and mechanical properties of cancellous bone from proximal femora of women with hip fracture and from female cadaveric controls, and found that the fracture group has distinctly lower BV/TV, Tb.N, and connectivity than the control group, and correspondingly has lower maximum modulus and ultimate stress [20]. In addition, their regression analyses suggested that the fracture and control groups exhibit quite different relationships between maximum modulus and bone volume fraction, and between maximum modulus and trabecula number, indicating their difference in the three-dimensional spatial arrangement of trabeculae. At the same time, the combination of μCT and the finite element method (FEM) provides us a powerful tool, μCT based FEM, to investigate the relationship between microarchitecture and mechanical properties of bone or other porous composites [14,19,21–23]. Recently, more detailed researches focused on the local microarchitectures showed that the percentage and orientation of the rod-like trabeculae in the cancellous bone, which is usually considered to be the useless small amount in volume or the deterioration shape of trabeculae brought by OP disease, have significant effects on the bone mechanical properties [24–28]. Stauber and Muller [24] proposed a novel framework for the element-based description of bone microarchitecture, decomposing cancellous bone into two basic volumetric elements: rods and plates. They applied the method to 328 samples of human cancellous bone and concluded that the strength of plate-dominant structure is determined by the major elements that span through the whole structure, while in the rod-dominant structure the strength is given by the arrangement and geometry of a whole set of elements. Liu et al. [27] quantified the contributions of trabecular rods of different directions to the elastic properties of human vertebral cancellous bone through μCT based FEM simulations and inferred that the transverse and oblique rods both make a significant contribution to all the moduli in and out of the transverse plane whereas the longitudinal rods only contribute to the moduli out of the transverse plane. They also demonstrated that the modulus reduction by removing these rods is beyond what could be explained by the corresponding bone volume loss, suggesting the involvement of architectural deterioration. Additionally, the computational simulations on the dynamic process of cancellous bone remodeling during menopause showed that microstructural plate perforation is the primary cause of menopausal bone loss, resulting in fewer and smaller trabecular plates and more but thinner trabecular rods [25,29,30]. μCT based FEM simulations were also conducted to investigate the roles of trabecular plates and rods in the failure behaviors of human vertebral cancellous bone under compression and revealed that trabecular rods play an important role in the failure initiation while trabecular plates play a critical role in determining the yield strength [26].

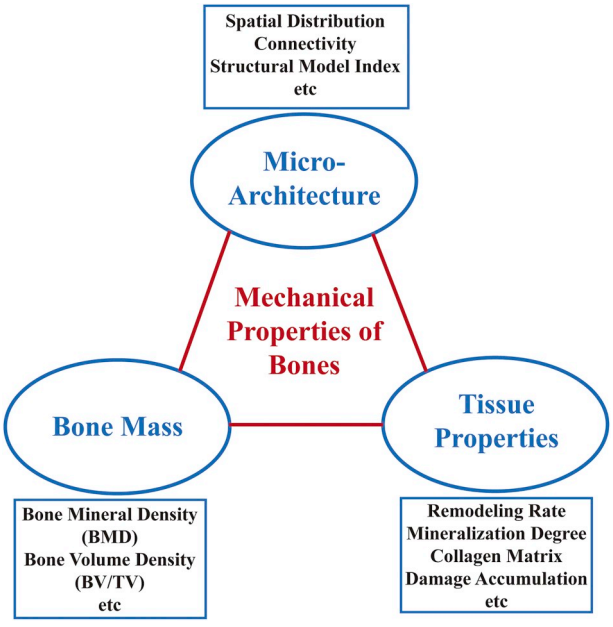

Fig. 1. Three-pillar framework to characterize the mechanical properties of bones.

There is no doubt that the microarchitecture has great impact on the mechanical properties of cancellous bone and osteoporotic bone exhibits distinct deterioration in microarchitecture. Nevertheless, it remains open to debate whether the properties of bone tissue material with OP are degraded significantly. Guo and Goldstein [31] measured the microscopic tissue elastic modulus and hardness of vertebral cancellous bone in ovariectomized rats with nanoindentation technique, and claimed that the estrogen-depletion induced osteoporosis did not change the elastic modulus and hardness of the surviving bone tissue at microscopic level. On the other hand, McCreadie et al. [32] examined the chemical composition of bone tissue obtained from women with and without osteoporotic fracture by Raman spectroscopy, and found that femoral bone tissue in fractured women had a higher carbonate/amide I area ratio and iliac crest cortical bone exhibited a higher carbonate/phosphate ratio than in unfractured women, indicative of the

deterioration of tissue material properties. Bone tissue material can be further viewed as a composite of mineral and collagen [16,33]. The high rate of remodeling and insufficient mineralization of course would reduce the mechanical properties of bone material. In addition, aging tends to decrease the elasticity of collagen and some diseases like diabetes and osteogenesis imperfecta would cause structural defects in collagen and then the mechanical deterioration of collagen [10,34]. Therefore, the deterioration of bone tissue materials is definitely an important aspect of concern when investigating the mechanical properties and fracture risk of whole bone [35,36].

As aforementioned the mechanical deterioration of OP bone is correlated to three major factors: bone mass, microarchitecture and tissue material properties. However, the separate influence and relative contribution of each factor has yet to be clarified. In the present paper, μCT based FEM simulations were conducted on ovariectomized and healthy mice models, the effects of bone mass, microarchitecture and tissue material properties on the mechanical deterioration of osteoporotic bone were investigated separately and respectively. The remaining of the paper is organized as below: Section 2 describes the materials and methods, Section 3 shows the results and relevant discussions, and the major conclusions are summarized in Section 4.

2. Materials and methods

2.1. Animal models and experimental samples

Eight weeks old female mice were randomly separated into two groups. One group was ovariectomized (OVX) and the other one was the healthy group for comparison. All the mice were housed in a regulated and standard specific-pathogen-free environment (24.0 ± 0.5 °C, 45%–50% humidity and 12/12 h light/dark illumination cycles), and allowed free access to tap water and diet. The animal house is managed by the Center for Animal Experiments, Wuhan University, in strict adherence to the AAALAC International standard for the production and usage of experimental animals. After twenty weeks, the OVX mice were carefully examined by in-vivo μCT scanner to make sure that the OP disease happened in the animal models, as shown in Fig. 2A. The OVX induced OP is analogous in pathogenesis to that widely seen in postmenopausal female and thus usually categorized into Type I osteoporosis. All the bones taken exactly at the same age of twenty weeks is in order to avoid the possible effect of age difference. Distinct bone loss was observed in five mice from the OVX group, two of them termed as severe OP with BV/TV less than 15% and the other three as mild OP with BV/TV less than 20%. The five OP mice from the OVX group and two healthy mice from the healthy group were sacrificed to collect their femurs as experimental samples. Then all the samples were fixed by 4% paraformaldehyde and kept under the room temperature. It is worth noting that the sample size is small (only two or three mice in each group) but enough, because the key research methodology in the current work is not statistics but a creative utilization of digital modeling and simulation techniques on these typical material samples. The new method as shown hereafter is more efficient and economical than the widely used statistics method which asks for a sufficiently large sample size.

All the animal experimental projects were approved by Institutional Animal Care and Use Committee, Wuhan University (No. 2019118). The procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. CT scan and 3D models of cancellous bone

The femur specimens were scanned by the high resolution μCT scanner (SkyScan 1176, Bruker, USA) at 9 μm nominal resolution with 8-bit grey level values. The X-ray source voltage was set to be 45 kV and the source current was 555 μA. An aluminum filter with thickness of 0.2 mm was used to remove noise. A rotation step as low as 0.3° was chosen to obtain the finest resolution that the scanner can provide. Based on the μCT data, 8-bit bitmaps of every cross section (thickness 9 μm) along the femur shaft were reconstructed using a modified Feldkamp algorithm [37] incorporated in the software Skyscan Nrecon (Bruker, USA). These 2D images were converted to 3D models with the help of the processing software Mimics Research (Materilise, Belgium). The standard grey value after calibration was set to make the trabeculae clearly outstand from the background. To avoid the influence of cortical bone and other tissues, the domain of cancellous bone was located at 450 μm below the growing plate. Five cubes of dimension 810 × 810 × 810 μm were taken as the volumes of interest (VOIs) from the cancellous bone domain of each femur, see an example shown in Fig. 2B, in which Z axis lies along the longitudinal direction of the femur shaft while X axis along the right-left direction and Y axis along the posterior-anterior direction. The VOIs were at least 90 μm distant from one to another so that the means and standard deviations from statistics of their properties can, respectively, better reflect the cancellous bone properties of each mouse and their fluctuations at different locations due to the heterogeneous nature of cancellous bone. Among all of the VOIs, three typical ones were selected to represent the healthy bone, mild OP bone and severe OP bone, respectively, as shown in Fig. 2C. Comparing the three typical structures, we can see that the healthy bone has a dense structure with a considerable number of thick trabecular plates, while the mild OP bone shows a distinctly higher porosity with thinning and perforation of trabecular plates, the severe OP bone becomes more porous with a number of trabeculae totally disappearing. Note that all the VOIs naturally have different bone mass, microarchitecture and tissue properties, and it is impossible to identify their individual effects through comparative studies on these natural VOIs. Hence, it is necessary to figure out a way to decouple the three factors, i.e., a way to let one factor vary while the other two fixed. To this end, a digital modeling parameter, grey value threshold is creatively used to artificially alter the bone mass of the typical VOIs while keep their microarchitectures

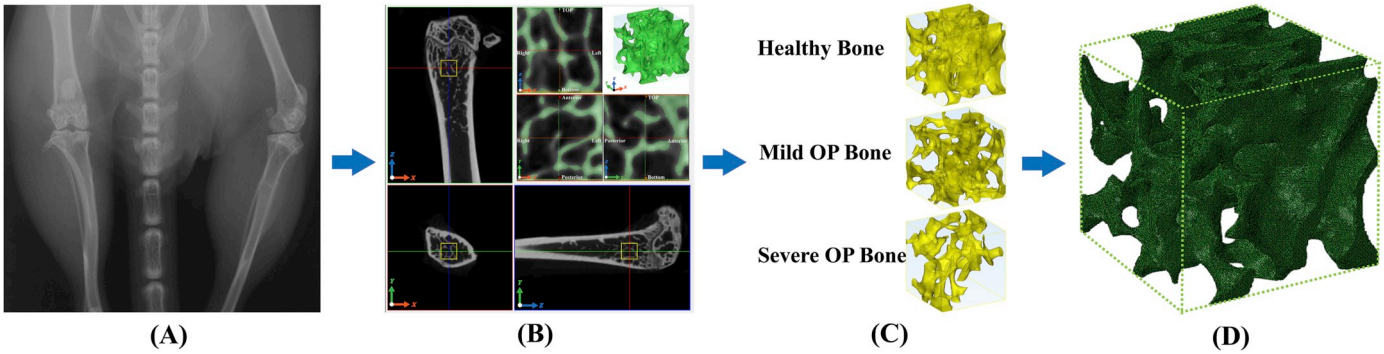

Fig. 2. (A) In-vivo μCT scan of mice; (B) Volume of interest (VOI) taken from the domain of cancellous bone in the femur samples and 3D microarchitecture rebuilt from μCT data; (C) Three typical VOIs: healthy, mild osteoporosis and severe osteoporosis; (D) An example of FEM model.

unaltered, and thus the two factors, bone mass and microarchitecture are successfully decoupled. The tissue properties represented by the material parameters can be independently manipulated in FEM simulations, and therefore can be decoupled from bone mass and microarchitecture with ease. The details about the decoupling strategies and studies will be described in Sections 3.2-3.4.

With the help of software Hypermesh (Altair Hyperworks Desktop, France), all the 3D structures of VOIs were meshed with C3D8 elements for further FEM simulations. A typical mesh model can be seen in Fig. 2D, in which the characteristic length of element is less than 8 μm compared to the 9 μm nominal resolution of μCT data so that the details of the 3D architectures are well kept. Each mesh model consists of more than 1 million elements.

2.3. Finite element method analyses

The Finite element method (FEM) simulations were conducted on the platform of Abaqus/CAE (Dassault SIMULIA, France). In the FEM simulations, a linear elastic model was first adopted for both the healthy and OP bone tissues, with a standard Young's modulus 20 GPa and Poisson's ratio 0.3 [38,39]. A small uniaxial compressive strain (0.001) was prescribed to each VOI by means of displacement control, and the reaction force can be computed. Let d, Δd, F denote the original dimension of VOI, the elongation and the reaction force, respectively. The average stress can be calculated by F/d^2, and the average strain by $\Delta d/d$. Hence, the effective or apparent Young's modulus of VOI can be obtained by the following equation

$$E = \frac{F}{d^2} \Big/ \frac{\Delta d}{d} = \frac{F}{d \cdot \Delta d}$$

With this approach, the effective Young's modulus along X, Y, and Z direction was, respectively, calculated for each VOI.

3. Results and discussion

3.1. Mechanical behavior of three typical bones

For each VOI, the BV/TV is defined as the ratio of bone volume over its apparent VOI volume. Statistics of the BV/TV data of VOIs give the means and standard deviations of BV/TV for every mouse, as shown in the Fig. 3A. Obviously, the healthy bone has a higher BV/TV than the OP bone, ~25% versus 10–20%. The 'Severe' and 'Mild' OP bone are with BV/TV ~10% and 20%, respectively. As aforementioned, the reduction of BV/TV of the mild OP bone is mainly attributed to the thinning and perforation of trabecular plates, without distinct alteration of structural topology, whereas the severe OP bone exhibits significant deterioration in structural topology with a number of trabeculae disappeared. FEM simulations on each VOI yield its effective Young's moduli along the X, Y and Z direction. Statistics of the modulus data of VOIs give the means and standard deviations of modulus for every bone sample. The effective Young's moduli of the bone samples against their mean BV/TV are displayed in Fig. 3B. Herein E_x, E_y and E_z are, respectively, the effective Young's modulus in the X, Y and Z direction, among which Z axis lies along the longitudinal direction of the femur shaft and the other two axes in the transverse plane as defined in Fig. 2B. It can be seen in Fig. 3B that OP disease generally induces significant degradation in the elastic property of cancellous bone. Fig. 3B also shows that the healthy cancellous bone is close to a transversely isotropic material, with E_x and E_y close to each other, and E_z about two fold higher than them (E_z:E_y:E_x = 2.2:1:0.7). The longitudinal superiority coincides with the major load-bearing function of femur bone along its shaft direction [40]. In contrast, the longitudinal superiority gradually vanishes and even reverses (i.e., E_z less than E_x and E_y) as OP develops from the mild (E_z:E_y:E_x = 1:1:0.64) to severe condition (E_z:E_y:E_x = 0.6:1:0.6), indicating the disruption of the load-bearing function of OP bones. In another word,

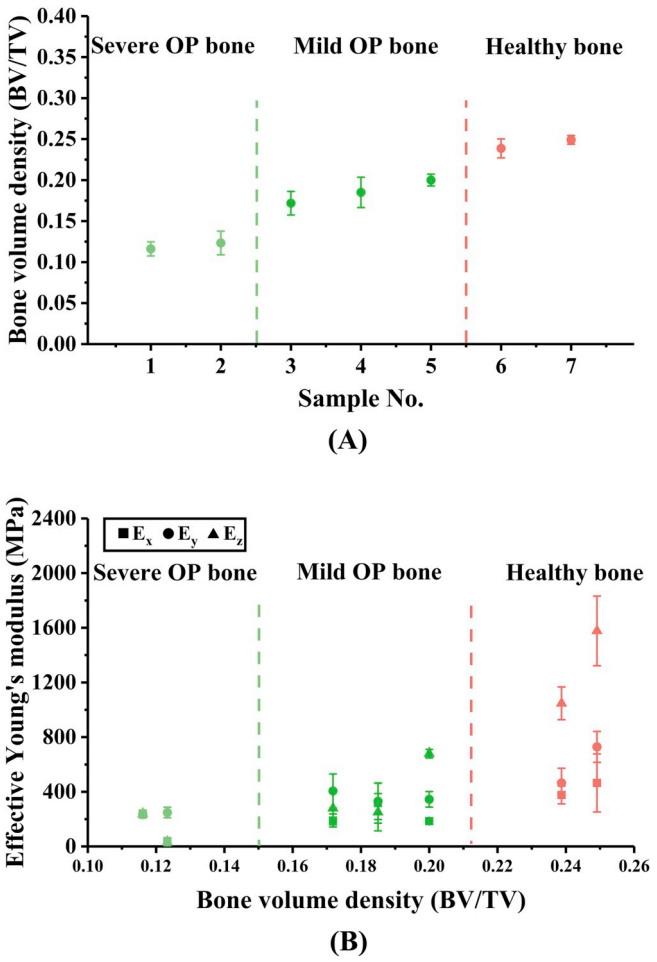

Fig. 3. (A) Bone volume density (BV/TV) and (B) Effective Young's moduli of different samples.

the degradations of stiffness in the longitudinal and transversal direction are not in proportion, that is difficult to well rationalize solely with the decrease of BV/TV. We suppose that the disproportionate degradation should be attributed to the deterioration of structural topology during the OP development.

3.2. Effect of bone mass

Definitely bone mass (here measured by BV/TV) is one of the most important factors in determining the mechanical properties of bone [19,21], with analogy to the role of volume fraction of reinforcements in defining the mechanical properties of composites [41]. However, in real cases of OP bone mass and microarchitecture usually change together and therefore it is difficult to study and clarify their individual effect on the mechanical deterioration of bones. As shown in Fig. 1, our 3D models are rebuilt from 2D grey-scale images from μCT scanning, and three typical bones are obtained with significantly different topological architecture and BV/TV. During the modeling process, a grey value threshold needs to be defined, by which the trabeculae can be distinguished from porosity in the μCT original bitmaps. Generally, the threshold should be determined by calibration so that the rebuilt model can well represent the real bone structure. However, properly changing grey value threshold provides us an efficient way to virtually alter the bone structure for investigating the separate effects of topological architecture and bone mass. As shown in Fig. 4, for each real and typical VOI (in yellow color) from Fig. 2B, we can change the grey value threshold linearly and slightly around the calibrated value so that the

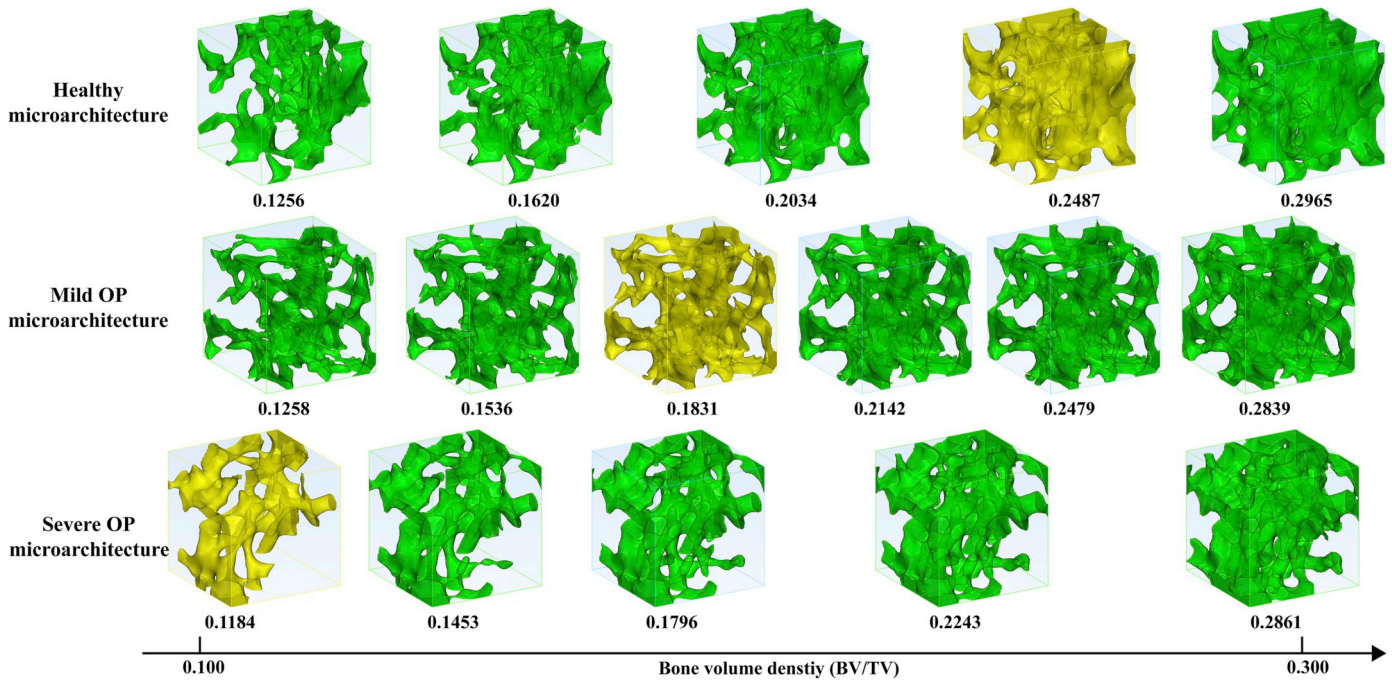

Fig. 4. A variety of bone volume densities acquired for the three typical microarchitectures (Healthy, Mild osteoporosis, and Severe osteoporosis) through altering in silico their thresholds of relative grey value.

trabeculae become a little thicker or thinner without changing their number [42], and consequently generate a set of artificial VOIs (in green color) with varying BV/TV but the same topological architecture. Conducting FEM simulations and comparative analyses on the mechanical properties of the three sets of VOIs, the individual effects of bone mass and microarchitecture can be identified in an ideally decoupled and separate way. Specifically, the influence of BV/TV can be figured out when we compare the mechanical properties of the VOIs in each row (with the same microarchitecture, varying BV/TV), and the influence of microarchitecture can be acquired when we compare the mechanical properties of the VOIs in each column (with similar BV/TV, different microarchitecture). The BV/TV ranges from 10% to 30% well covering the typical values of healthy bone, mild OP bone and severe OP bone, and thus we can check with healthy microarchitecture how sole bone loss would deteriorate the mechanical properties of bones, and with OP microarchitecture how sole bone gain would recover the mechanical properties of bones. Answers to these questions are critically important to the diagnosis and treatment of OP.

The effective Young's modulus in X, Y, Z directions have been calculated via FEM simulations for all the VOIs in Fig. 4. Fig. 5A–C plot the effective Young's modulus against BV/TV for the healthy microarchitecture, mild OP microarchitecture and severe OP microarchitecture, respectively. It is obvious that the effective Young's moduli are always going up quickly with the bone volume density, somehow like an exponential function, regardless of the loading direction and the type of microarchitecture. For the healthy microarchitecture, the longitudinal modulus changes significantly faster than the transversal moduli, and the longitudinal modulus is always larger than the transversal. This implies that for the healthy bone microarchitecture the longitudinal stiffness is more sensitive to the bone volume density, but it is robust enough to keep its functional feature of longitudinal superiority for a quite large range of bone volume density. For the mild OP microarchitecture, the moduli in all the three directions vary in a similar fashion with respect to the bone volume density, but the modulus in the Y direction slightly stands out instead of that in the Z direction, indicating the breakup of longitudinal superiority. For the severe OP microarchitecture, the plots of moduli in the X and Z direction against the bone volume density are close to each other, while the curve of modulus in the Y direction versus bone volume density goes up higher and faster.

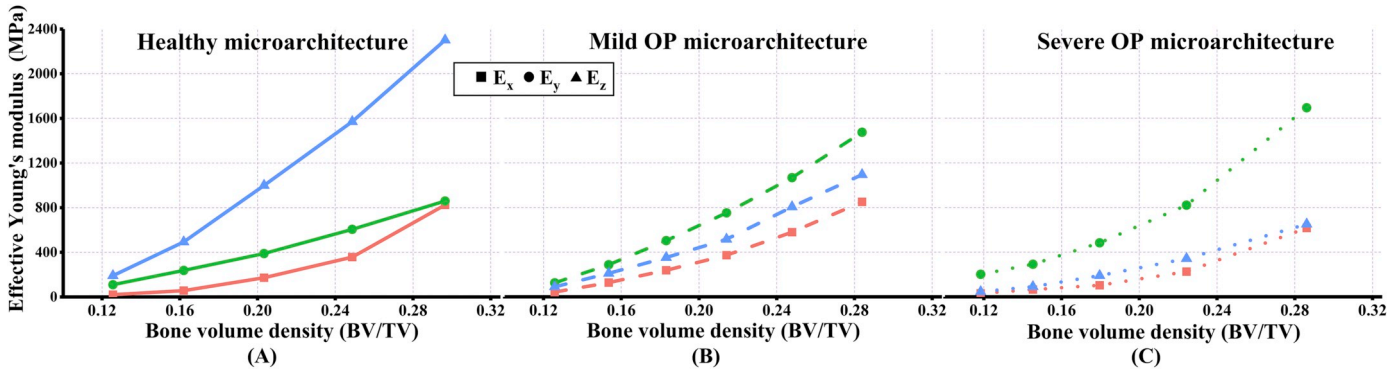

Fig. 5. Effective Young's modulus in the X, Y, Z direction varying with respect to bone volume density for the three typical microarchitectures: (A) Heathy bone, (B) Mild osteoporotic bone and (C) Severe osteoporotic bone.

3.3. Effect of microarchitecture

The microarchitecture represents the three-dimensional spatial distribution of bone tissue material and defines the directional (isotropic or anisotropic) features of the mechanical properties of bones. Through long term of evolution, the healthy bone architecture is optimized mainly for its mechanical functions such as supporting body weight and locomotion [43]. To better show the anisotropic feature of different microarchitecture, the modulus ratios E_z/E_y and E_x/E_y are plotted with respect to the bone volume density in Fig. 6. It can be seen clearly that the healthy architecture provides an important feature of longitudinal superiority in the effective Young's moduli of bones, which well matches to the major function of long bones, resisting loading such as body weight and impacts from walking or running mainly along the longitudinal direction. Even if the bone volume density is reduced, the longitudinal superiority is still well kept for the healthy microarchitecture, for instance E_z:E_y:E_x = 1.7:1:0.24 even when BV/TV = 0.1256. In contrast, OP does not only reduce the bone mass but also hurt the functionally optimized microarchitecture. The effective Young's modulus of OP bones in every direction is distinctly lower than their counterpart of healthy bones, and the longitudinal direction is weakened so much that its modulus becomes smaller than the transversal direction (i.e., E_z:E_y < 1, see also Fig. 3B), thus the longitudinal superiority disrupted. Even if the bone volume density is increased to the healthy level (~25%), the effective Young's modulus in the longitudinal direction (also the major loading direction) for OP microarchitectures is still much below the healthy level (515 MPa, 342 MPa versus 1571 MPa for mild and severe case, respectively). At the same time, their effective Young's moduli in the transversal directions become unnecessarily high, surpassing the healthy level (E_z:E_y = 0.7:1, 0.4:1 for mild and severe case, respectively). As the principal direction of major loadings on long bones is along their longitudinal axis, the longitudinal superiority in mechanical properties is evidently beneficial to their load-bearing function, and the abnormal deviation from the longitudinal superiority is certainly harmful to the mechanical function and raises the fracture risk of bones. On the other hand, the results also suggest us that the clinical treatment on osteoporosis should not only focus on the regaining of bone mass, but also pay more attention to the recovery of inner microarchitecture. The longitudinal superiority can be taken as an important index measuring the healthy level of microarchitecture of cancellous bones.

3.4. Effect of tissue property

As mentioned in the introduction section, there are some studies reporting that the tissue material consisting the cancellous bone would degrade in mechanical properties together with the development of OP disease, even though some other studies suggested it may not. However,

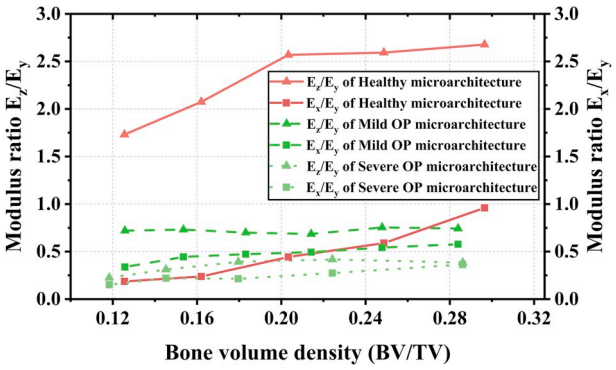

Fig. 6. Ratios of the effective Young's modulus E_z/E_y and E_x/E_y varying against the bone volume density for the three typical microarchitectures: Heathy bone, Mild osteoporotic bone and Severe osteoporotic bone.

it is still desirable to investigate how it would affect the mechanical properties of cancellous bones if the bone tissue material varies in mechanical properties. For simplicity, the bone tissue material in the paper is always regarded as homogeneous, isotropic, and linear elastic. In this scenario, the mechanical variance of bone tissue can be reflected mainly by the change of its Young's modulus. The Young's modulus of bone tissue under the healthy condition is set to be 20 GPa [38,39,44], a typical value found in the literatures. Around the standard value, a range of Young's modulus from 16 GPa to 24 GPa is studied related to the effective Young's modulus of the typical VOIs of cancellous bones shown in Fig. 2C. The effective and apparent strain of VOI models is still prescribed up to 0.001 by uniaxial displacement control, fitting well to the linear elastic and small deformation situation. The simulated effective Young's moduli of the three typical VOIs are plotted versus the variance of bone tissue Young's modulus in Fig. 7A–C, respectively. It can be seen that the effective Young's moduli in all directions of all the three typical VOIs are positively and linearly related to the Young's modulus of bone tissue. As expected is that the bone tissue property change does not alter the anisotropic feature of the mechanical properties of cancellous bones. The slopes of the plotted lines of effective moduli versus tissue modulus for the severe OP VOI are especially small regardless of the direction, which indicates that the stiffness of severe OP cancellous bones is insensitive to the tissue material modulus due to their such a low connectivity and integrity in microarchitecture. Comparing the lines of the healthy and mild OP VOIs, we can find that their transversal moduli vary against the tissue property in a similar way, slightly sensitive to the tissue Young's modulus, but the longitudinal modulus of the healthy VOI is distinctly more sensitive to the tissue Young's modulus. Interestingly, even when the Young's modulus of bone tissue reduces by 20%, from the standard value 20 GPa to 16 GPa, the longitudinal stiffness of healthy VOI is still much larger than that of OP VOIs with the standard value. Therefore, we can infer that the influence of tissue material degradation in OP bone mechanical properties may not be as significant as those of bone mass and microarchitecture.

To summarize the effects of bone mass, microarchitecture and tissue property, there are two points worth to be emphasized. First, sole bone loss without destroying the healthy microarchitecture would decrease the effective stiffness of cancellous bone but would not hurt its functional feature of longitudinal superiority. Second, sole bone mass gaining for the OP microarchitectures would increase the effective stiffness of cancellous bone, but cannot recover to the healthy condition; especially, the longitudinal superiority is critically important for bones' load-bearing functionality but not recovered. Correspondingly, the following suggestions can be made on the diagnosis and treatment of OP. First of all, more attentions should be paid to the microarchitecture in the OP diagnosis and treatment, especially taking into consideration that the importance of bone mass has been widely recognized and the relevant measures have been developed and used very well in clinics. Secondly, more efforts should be made to recognize the critical features of microarchitecture of healthy cancellous bones, for example the longitudinal superiority emphasized in the present paper which may be used as an important measure to characterize the deterioration of OP cancellous bones in microarchitecture. Thirdly, early diagnosis and treatment of OP is particularly important before significant microarchitecture deterioration. Forth, the microarchitecture recovery should be sought together with the bone mass regaining to guarantee a better recovery of the mechanical property of bones during OP treatments.

4. Conclusions

In the paper, we have used the ovariectomized mice to establish the animal models of type I osteoporosis (OP). Based on high resolution μCT scanning data, 3D digital models were rebuilt for FEM simulations to investigate the mechanical degradation and relevant factors of cancellous bones with different OP conditions. To better describe the mechanical deterioration of osteoporotic bones related to the possible

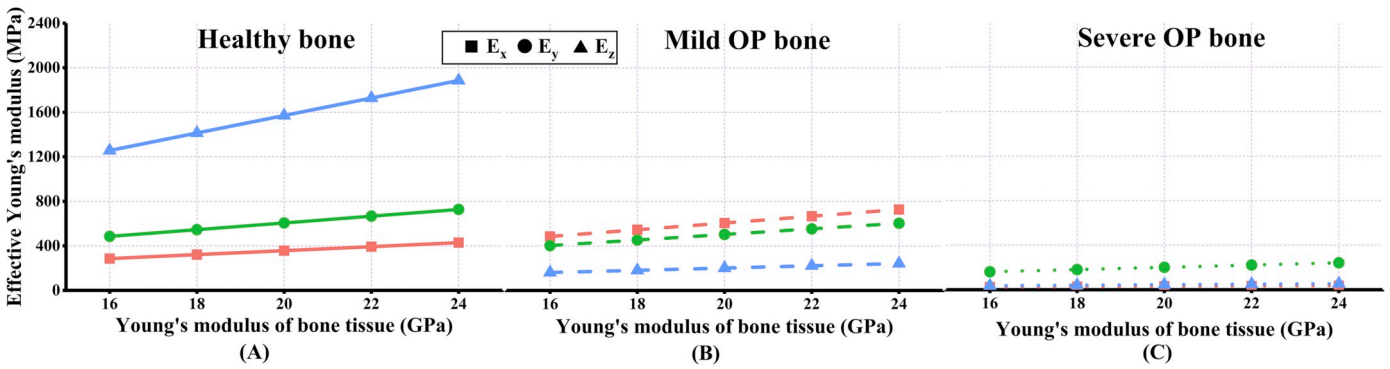

Fig. 7. Effective Young's modulus of different bones changing with the tissue property: (A) Heathy bone, (B) Mild osteoporotic bone and (C) Severe osteoporotic bone.

factors, we proposed a three-pillar framework based on the theory of composite mechanics, "bone mass-microarchitecture-tissue properties", instead of the existing "bone mass-bone quality" framework to avoid the ambiguity of the bone quality concept. According to the three-pillar framework, we first compared the bone volume density, microarchitecture and mechanical properties of three typical cancellous bones, namely, the healthy, mild OP and severe OP bones, and then we decoupled the three major factors, bone mass, microarchitecture and tissue properties, through digital modeling and FEM simulations to probe into their individual and integrative effects on the mechanical properties of healthy and diseased bones. The results and findings lead to the following major conclusions:

1) Compared to the OP bones, the healthy bone has an obviously larger bone volume density (BV/TV) and a better microarchitectural topology, and so exhibits a higher stiffness and the longitudinal superiority which is a critical feature maintaining the load-bearing function of long bones.
2) Bone mass individually has significant influence on the magnitude of the effective Young's moduli of cancellous bones while microarchitecture plays a critical role in defining the anisotropic features of elasticity of cancellous bones.
3) OP not only reduces the bone mass but also damages the optimized microarchitecture of healthy cancellous bones. Thus, it breaks down the longitudinal superiority of cancellous bones and increase their fracture risk under daily physical loadings.
4) The sole contribution of the tissue material degradation to the mechanical deterioration of osteoporotic bones is not as much as those of bone mass and microarchitecture, even if there are really a slight reduction in the mechanical properties of osteoporotic bone tissue compared to those of healthy bone tissue.

At the end, it is worth noting that even though the current work just focuses on the elastic properties of osteoporotic cancellous bones, many other aspects of mechanical properties of bones such as strength, ductility and fracture toughness can also be studied in the three-pillar framework.

Conflicts of interest

None to disclose.

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