

# THE EFFECTS OF COD BONE GELATIN ON TRABECULAR MICROSTRUCTURE AND MECHANICAL PROPERTIES OF CANCELLOUS BONE<sup>★★</sup>



Renhan Huang<sup>1</sup>    Qiguo Rong<sup>1\*</sup>    Xiaolong Han<sup>2</sup>    Yong Li<sup>3</sup>

*(<sup>1</sup> College of Engineering, Peking University, Beijing 100871, China)*

*(<sup>2</sup> China Astronaut Research and Training Center, Department of Space Food and Nutrition,  
Beijing 100094, China)*

*(<sup>3</sup> School of Public Health, Peking University, Beijing 100083, China)*

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**ABSTRACT** Osteoporosis is a common clinical complication of post-menopausal women and the elderly and can significantly complicate the severity of bone fragility. The purpose of this study is to investigate how cod bone gelatin administration influences trabecular biomechanical properties after ovariectomy. Both biomechanical properties and trabecular microarchitectures were evaluated for cancellous bone samples from female ovariectomized rats, which were either sham-operated or treated with marine peptide (0.75, 1.5, 3.0, 6.0 g/kg body weight) for 90 days. The results have confirmed that cod bone gelatin treatment is effective in the prevention of mechanical property loss by preserving bone mass and trabecular architecture.

**KEY WORDS** osteoporosis, cod bone gelatin, trabecular microarchitecture, finite element analysis

## I. INTRODUCTION

Osteoporosis affects predominantly postmenopausal women and is one of the most common bone diseases caused by unbalanced bone remodeling process, with higher bone resorption than bone formation. Postmenopausal osteoporosis is characterized by low bone mass, microarchitectural deterioration, and decreased bone strength that lead to an increase in fracture risk. According to a study about Chinese people ages above 40, the overall prevalence rate of osteoporosis is 16.1% (11.5% among males and 19.9% among females)<sup>[1]</sup>. Though the prevalence of osteoporosis among Chinese population remains low compared to other Caucasian populations, it increases with aging and changing lifestyles. Several types of treatment for osteoporosis are currently available, including bisphosphonates, estrogen agonist/antagonists, parathyroid hormone, estrogen therapy, hormone therapy, etc. However, some agents have toxicity or cause intolerance reactions in individual patients. Thus, much attention has been paid to compounds that provide greater symptomatic relief with less overall toxicity.

Nowadays, there is a growing interest in seeking alternative therapeutic agents in the treatment of osteoporosis. Gelatin is an attractive candidate for its high level of safety and overall lack of toxicity. Adam et al.<sup>[2]</sup> found that orally administrated gelatin improve bone collagen metabolism in osteoporosis

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\* Corresponding author. E-mail: qrong@pku.edu

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patients. Recently, Han et al.<sup>[3]</sup> indicated that the cod bone gelatin treatment can prevent bone loss by decreasing bone resorption in ovariectomized rats.

Cancellous bone has a cellular structure made up of bars and plates that plays an important role in the load bearing function of the skeleton, and is significantly influenced when diseased. In osteoporosis, cancellous bone is more severely affected than cortical bone. Much as mechanical properties depend primarily on bone apparent density, the role of microarchitecture cannot be ignored. In most postmenopausal women, estrogen deficiency increases the rate of remodeling and the imbalance in the bone multicellular unit which accelerates bone loss and structural decay. Osteoporosis medication is able to protect bone architecture by reducing the rate of bone remodelling, promoting the completion of remodelling and reducing the depth of resorption. The aim of this study is to find out whether the cod bone gelatin treatment improves the biomechanical properties of cancellous bone after ovariectomy. The microstructural changes of cancellous specimens were evaluated by the mean intercept method while the mechanical behaviours were studied by the finite element method.

## II. VARIATION IN MICROARCHITECTURE

### 2.1. Animal Procedures

Three-month old female Sprague Dawley rats were housed at  $21 \pm 2$  °C, humidity  $55 \pm 10\%$ , and a 12 h light/dark cycle. All animals were supplied with water and AIN-93M diet. After 14 days of equilibration, these animals were either randomized (OVX) or sham-operated (SHAM) on. Bilateral ovariectomies were performed under sodium pentobarbital anesthesia. Sham operations were performed by exteriorizing and replacing the ovaries. OVX rats were divided into 5 groups: a distilled water-treated group and groups treated with cod bone gelatin at 0.75, 1.5, 3.0, 6.0 g/kg weight, respectively. Gelatin was prepared from fresh cod (*Eleginus gracilis*) backbone according to the method described in a previous study<sup>[4]</sup>. The cod bone gelatin dose was mixed with water as a vehicle and administered orally daily. After surgery, the ovariectomized (OVX) rats were pair-fed with the SHAM-operated rats to prevent postovariectomy hyperphagia. Food consumption was recorded weekly for the SHAM group and this amount of food was then fed to the OVX rats during the following week. At the end of treatment, the animals were sacrificed by an overdose of sodium pentobarbital. Tibias were removed, freed of soft tissue and kept at  $-20$  °C for later analysis. All experimental designs and procedures were performed with the approval of the Peking University Animal Ethics Committee.

### 2.2. Micro-CT Scan of Tibias

The proximal tibia metaphysis is the most common skeletal site selected for analysis of cancellous bone for histological and structural analyses. The right tibia metaphysis was scanned by a Micro-CT 40 scanner (Scanco Medical, Bassersdorf, Switzerland). The scan region consisted of the central-most region of the tibia with a thickness of 2.00 mm. The 1 mm<sup>3</sup> volume of interest (VOI) was selected from the region 1.5 mm distal from the growth plate.

### 2.3. Change of Microarchitecture

For this preliminary study, only one specimen from each experimental group was randomly chosen to study the microstructural and mechanical changes. The three dimensional trabecular microstructures of all VOIs were reconstructed based on the micro-CT data as shown in Fig.1. The sparsely distributed trabeculae in the ovariectomized rat contrast sharply with the normal trabecular structure. The cod bone gelatin treatment obviously influences bone density and porosity.

Figure 2 shows the total surface area and volume of trabeculae in VOIs. A significant increase in surface area and volume was observed in 3 g/kg and 6 g/kg specimens as compared to the OVX specimen. On the contrary, low dose specimens (0.75 g/kg and 1.5 g/kg) were not significantly different from the OVX specimen. It may hint that a minimum dose of cod bone gelatin is necessary to improve the bone mass. If the density of bone tissue is considered constant, the bone volume of a VOI can then be considered as the same as the bone apparent density.

In addition to the bone density, the spatial distribution and connectivity of trabeculae play also a key role in bone quality. Not all trabeculae are connected as a whole structure. The trabeculae with one of its ends falling in the VOI will not contribute to the load bearing ability. The VOI of the SHAM

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