# Determination of micromechanical properties of healthy and defective chicken bone

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**Abstract**
The mechanical and micromechanical properties of cortical hen bones are influenced by numerous factors. Their knowledge is important for both, scientific and economical reasons. This paper presents the results of nanoindentation experiments (with standard Berkowich tip) performed on two types of tissue samples: femoral bone of healthy laying hen and laying hen with defective calcium metabolism. The results have shown the significant difference between tested materials in both, elastic modulus and microhardness values. The difference represented 11 and 12 % respectively (the higher value belong to healthy tissue). The shape of *E* and *H* histograms revealed certain degree of bimodality, which could be related to the prefferential collagen fiber orientations in the tested area. The other parameters such as porosity were also monitored and evaluated. The approach, data processing, and results evaluation were compared with studies focused on human bones. It was found that well described and deeply studied mechanism occurring in human tissues can be applied to chicken bones.

***Key words:*** *chicken bone, micromechanical properties, nanoindentation*

## Introduction

Knowledge of mechanical and micromechanical mechanism connected with chicken bones is very important, namely for understanding the process and reasons of bone breakages and their relation to calcium (or other elements) content. Composition of the chicken bone tissue (and other species bones as well) is extremely complex compared to most engineering composites (Ascenzi, 1988). Different factors such as diet, breeding conditions, genetic nature, and/or hen breed were monitored as determining factors. The differences between individual birds in terms of e.g. bone fracture incidence can not be explained by a sole factor. Differences can occur due to calcium metabolism, bone structure, or simply due to the body weight differences. A combination of factors is most likely involved (Clark et al., 2008). Micro mechanical properties significantly affect the mechanical behaviour of the whole bone, as it was documented in number of works (e.g. Hengsbergera et al., 2003) and their detailed determination and interpratation is thus needed. Precise and successfully tested procidere suitable for such task is the nanoindentation.

Nanoindentation enables measurement of the mechanical properties of bone material at a spatial resolution similar to that of the tissue-level structural features in bone. In nanoindentation, a small probe with nano to micro-meter dimensions contacts a flat, prepared surface of a material where the resulting force and contact depth (i.e., displacement) data enable calculation of elastic, plastic, and viscous material properties (Oliver and Pharr,1992; Oyen and Cook, 2003; Olesiak et al., 2010). Indentation is a multiaxial test that produces measured properties which are influenced by the material itself as well as inhomogeneities contained withina 3-D volume of high stress located underneath the indenter tip (Bushby, 2001).

## Material and methods

### Chicken bones

Two types of bone tissues were evaluated. Both belonged to Rhode Island Red (RIR) laying hen. One of the tissues was extracted from the cortical part of a femoral bone of clinically healthy hen (51 weeks old) with the incidence of cracked eggs lower than 2 % (further denoted as Healthy-series). The second one was extracted from the cortical part of a femoral bone of a hen with calcium metabolism defect (denoted as Ill-series). This defect was shown by a high presence of cracked eggs (more than 20 %). The similar specimens were tested in research presented in Severa et al (2011). The schematic of different anatomical bone elements are shown in Fig. 1.

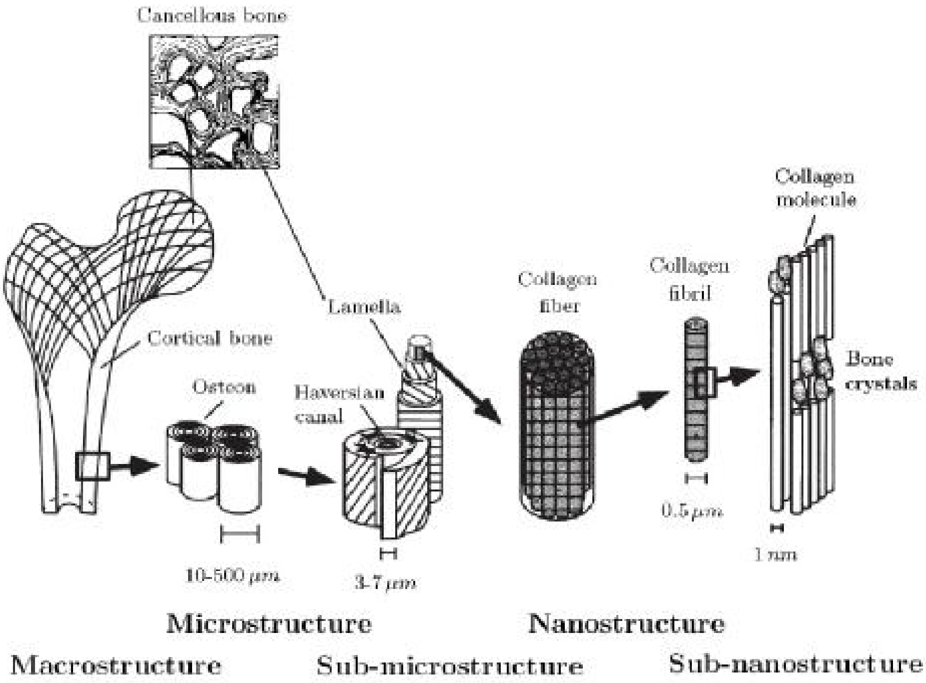


*Figure 1 Schematic of cortical bone anatomy (adapted from Rho et al)*

**Preparation of specimens**

The femoral diaphysis of a mature hen (RIR) were dissected and dried for 48 hours at room temperature. Effect of the bone drying and affecting the values of Young's modulus and hardness was documented for bovine bones but not for hen's bones so far. The samples were milled down to a cylindrical shape of 10mm in height, their main (cylindrical) axis being aligned with the longitudinal direction of the diaphysis. The specimens geometry and different stages of the testing procedure are shown in Fig. 2, 3, and 4. After this preparatory step, the specimens were embedded into metacrylate tablet. The specimens were cold-prepared (the structure was not thermally affected). Commercially available two-component resin was used for metacrylate mixture preparation and the specimens were left to dry and cure for 8 hours. The tablets were polished in order to achieve flat surface with maximum roughness of 10-20 nm. Similar procedure was successfully used by Severa et al (2011).

*Figure 2 Macroscopic view of femoral bone specimen*

*Figure 3 Bone rings intended for embeding into metacrylate*

**Testing conditions**

Nanohardness tester (CSM Instruments, Switzerland) was used to perform the experiments. Berkovich tip of standard geometry was brought to the sample surface, producing a series of imprints. Influences of the tip geometry, contact depth, and contact area on nanoindentation properties of the bone were broadly discussed in literature, and the results were used for the configuration of presented experiments. The indenter has a nominal tip radius of R ≈ 50 nm and a half-angle apex of $q$ = 65.27°. The bone fragments were loaded in directions perpendicular to the cross-sections. Load vs. depth of penetration was measured throughout the whole procedure of loading, holding, and unloading. The load-controlled test was performed using the standard trapezoidal loading diagram as follows: linear loading (60 mN/ min) up to the peak force (5 mN), then a 10 s holding period at the maximum force and linear unloading (60 mN/min) to zero force level (Fig. 4). Each parin pari-section was covered with a grid of 80 indents with 12 µm paring. Similar experimental procedure and set-up was used e.g. by Severa et al.

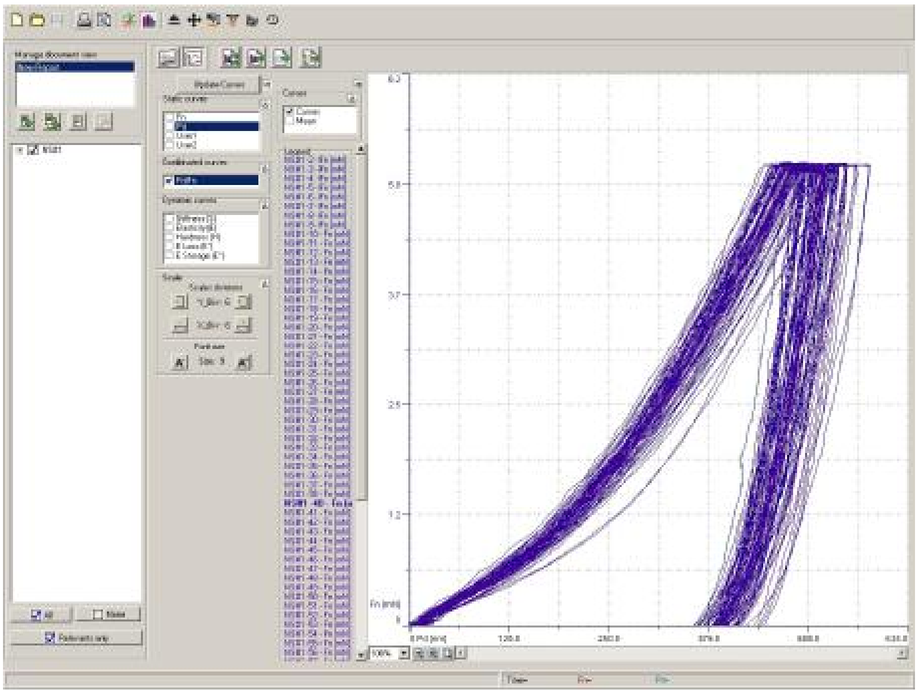

*Figure 4 Trapezoidal loading diagram*

## Results and Discussion

Two parameters were evaluated: elastic modulus (*E*) and indentation hardness (*H*). Standard approach with cycling loading and unloading was used. The elastic parameters show high scatter, but easily recognizable decrease in both *E* and *H* can be observed for series determined for defected bone. Histogram of elastic moduli is shown in Figure 5.

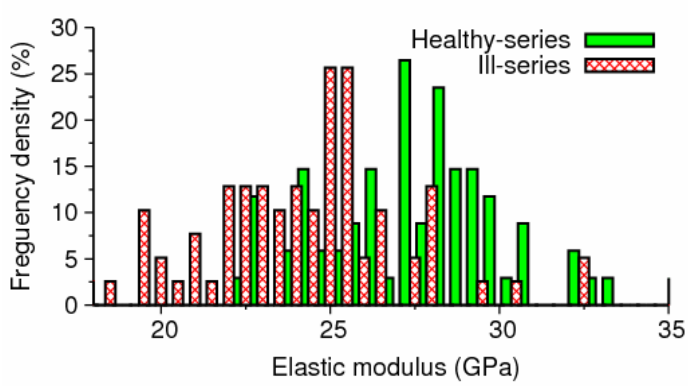


*Figure 5 Elastic moduli histograms for healty and ill-series.*

It was found that healthy bones are characterized by higher elastic modulus *E* and indentation hardness values *H*. It points to the fact that both elasticity and strength parameters (that are related to *H*) are afected in Ill-series. In case of non-defective tissue, following values were determined as: E=27.5±2.8 GPa, H=0.99±0.11 GPa, while in case of defective tissue as: E=24.5±3 GPa, H=0.88±0.07 GPa. Generally speaking, the values measured on the defective bone are approximately 11 % lower. Optical microscopical analysis revealed that healthy bone tissue contained larger pores in comparison with defective one. There are different methods of porous materials evaluation, e.g. with relation to their moisture content (Hlaváčová, 2005). The example of optical microscopy snapshot and indent capture is shown in Fig. 6. Interpretation of this phenomena requires much larger investigation and experimental data background.

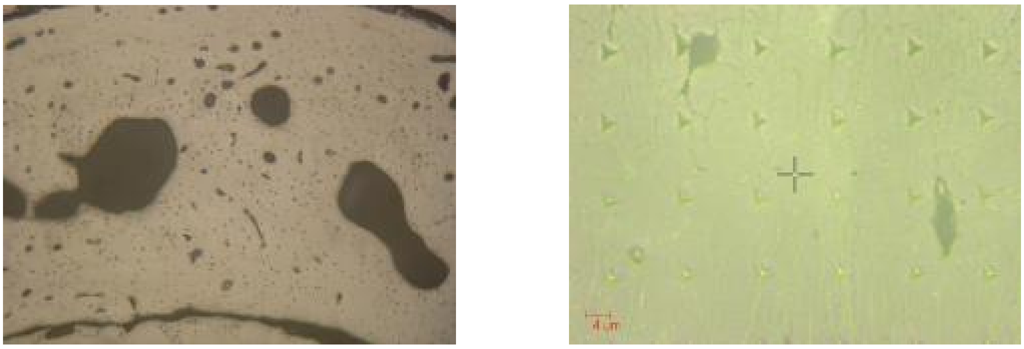

*Figure 6 Optical microscopy snapshot and indent capture*

The shape of the *E* and *H* histograms shows also on some kind of bimodality in the case of Healty-series which could be related to the prefferential collagen fiber orientations in the tested area. Despite this fact, presented study was focused on the evaluation of average properties in one direction only to give overall insight into the problem. An additional information could be received e.g. by determining the electric and/or dielectric properties and method described in Hlaváčová (2003).

## Conclusions

The composite mineral and organic structure of the hen bone does not deform plastically, rather bone is more likely damaged during nanoindentation. This fact was documented in literature for human bone tissues. This research quantified the differences between two types of chicken cortical bones (healthy and defect one). The values of elastic modulus and hardness were cca 11 % lower in case of defected tissue samples. The data revealed and confirmed the fact that mechanisms described for human tissues can be largely adopted and used for detailed further research of chicken bones as well.

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