# NANOINDENTATION STUDY OF NORMAL AND OSTEOPOROTIC BONES

C.T. Lim, B.H.B. Omar and J.C.J. Goh [1]
Division of Bioengineering & Department of Mechanical Engineering, [1] Department of Orthopaedic Surgery,
National University of Singapore, Singapore

## ABSTRACT

This paper investigates the nanomechanical properties of both normal and osteoporotic rat femur bones using the nanoindentation technique. Here, the Young's modulus and hardness were determined at specific locations within the bone. Microtomography was also used to evaluate the morphological differences between the bones. In general, the mechanical strength of osteoporotic bone was found to be lower than normal bone and the trabecular part more porous. The spatial resolution with which testing was carried out mattered in identifying the mechanical property and morphological differences at the micron and nanometer scale between normal and osteoporotic bones. This can help in the research on the diagnosis of osteoporosis or testing the efficacy of certain treatment methods.

## 1 INTRODUCTION

Osteoporosis is a major cause of morbidity, mortality and medical expenses worldwide. This increase is expected to continue with an aging population and as more people adopt a sedentary lifestyle and a poor diet. An osteoporosis sufferer has a lower bone mass and experiences structural deterioration of the bone tissue. As such, an osteoporosis patient can be susceptible to fractures even from activities such as sneezing or giving a hug. The question arises whether this structural deterioration is manifested as a change in the arrangement of the matrix that makes up the bone or just an intrinsic weakening of the individual bone filament. This paper seeks to investigate this by examining the nanomechanical properties of both normal and osteoporotic bones using the nanoindentation technique.

## 2 MATERIALS AND METHODS

Ten female Wistar rats were used in this study. An ovariectomy was performed on five of these rats. The bones were stored with flesh at -20ºC to maintain their mechanical properties (Cowin [1]). Only the femurs were used in the experiments. The left femurs were first imaged using microtomography. The right femurs were tested using the nanoindentation technique. To prevent demineralization of the bone, the soft tissue was removed using a scalpel instead of immersion in potassium hydroxide.

For nanoindentation, the right femur bone was embedded in acrylic mould 28 mm in diameter, up to 1 cm from the distal end. This allowed the femur to be clamped so that sawing and polishing could be performed. The femur was cut longitudinally 2 mm from the centre of the shaft using a diamond saw. The surface was polished with grit paper and alumina slurry. Following that, another cut is made 2 mm from the polished surface. The sample was then attached to a glass slide using cyanoacrylate adhesive. A Triboscope indenter mounted on a Digital Instrument Dimension 3100 atomic force microscope was used for the nanoindentation tests. A berkovich tip was used for indentation and imaging. For every sample, eight indents were made on the cortical bone. Figure 1 shows the location of the indents.

The load was increased linearly to a maximum load of 2000µN over a period of 10 seconds. The load is then held constant for 15 seconds before it is reduced to zero over a period of 10 seconds. The 15-second hold period is essential as bone materials are known to possess rate dependent behavior. The Young's modulus and hardness were then calculated from the load-displacement data (Oliver et al [2]).

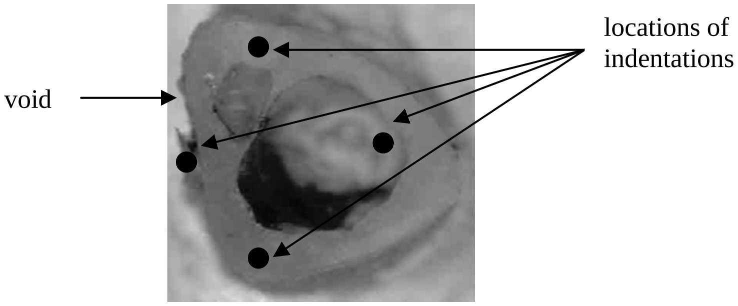


Figure 1: Location of indents

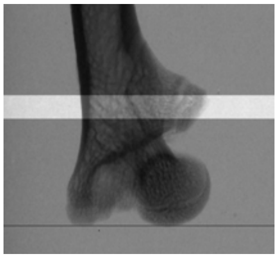


Figure 2: Slice of bone analysed using microtomography

A Skyscan 1076 micro-CT was used for the microtomography experiments. All the scans were carried out at 18µm resolution at the proximal end as shown in Figure 2. The images obtained from microtomography are then used to form a three-dimensional model. From this model, the trabecular thickness and trabecular separation of the sample was obtained. The statistical software Minitab 14 was used to conduct a one-way analysis of variance (ANOVA) on the results. All tests were done at 0.05 level of significance.

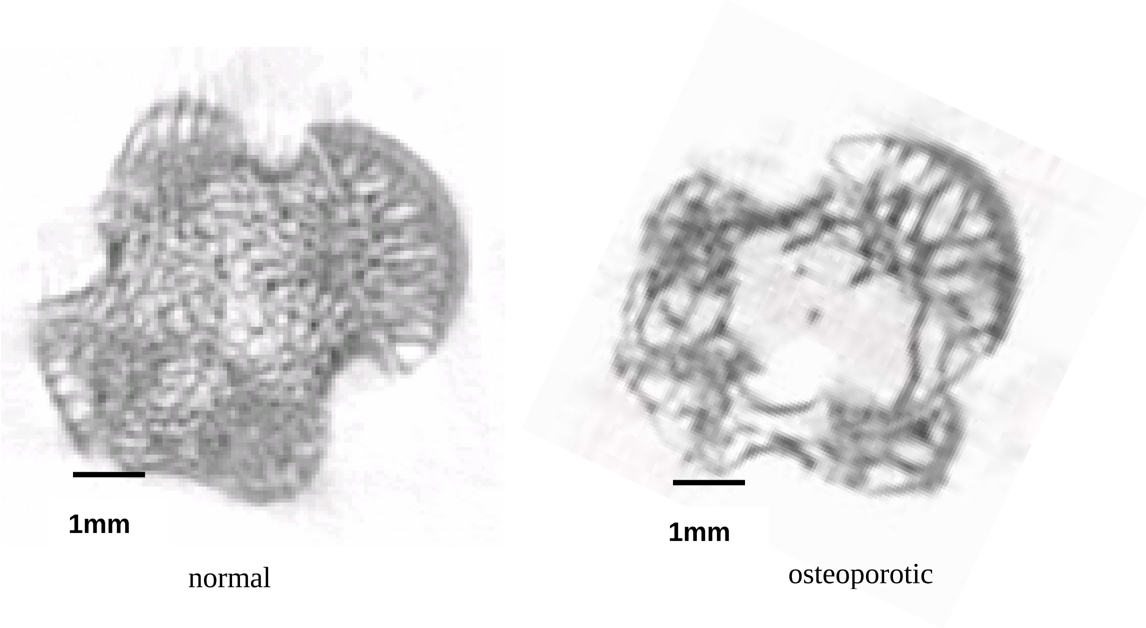
## 3 RESULTS AND DISCUSSION

The results of the nanoindentation tests are shown in Table 1. The values of Young's modulus and hardness for the normal bones are slightly higher than that reported by (Jamsa et al. [3]) by 21 and 38%, respectively. This could be due to the rats used in this experiment being older (6 weeks) than those used by Jamsa (2 weeks). The different strain of rat or diet could have also resulted in the discrepancy in the strength values. The values of Young's modulus and hardness of osteoporotic bone were found to be 19 and 40%, respectively and were lower than that of the normal bone. Osteoporosis has an obvious effect on the Young's modulus and hardness of the rat femurs.

Table 1: Mean values of Young’s modulus and hardness from nanoindentation

| **Normal** | | | **Osteoporotic** | | |
|---|---|---|---|---|---|
| **Rat** | **Young’s modulus (GPa)** | **Hardness (GPa)** | **Rat** | **Young’s modulus (GPa)** | **Hardness (GPa)** |
| **N1** | 23.54 | 1.23 | **O1** | 19.73 | 0.76 |
| **N2** | 22.15 | 1.14 | **O2** | 18.75 | 0.66 |
| **N3** | 25.88 | 0.90 | **O3** | 18.30 | 0.61 |
| **N4** | 24.26 | 1.19 | **O4** | 19.46 | 0.69 |
| **N5** | 22.24 | 1.06 | **O5** | 19.16 | 0.54 |
| **Mean ± S.D** | 23.61 ± 1.55 | 1.10 ± 0.13 | **Mean ± S.D** | 19.08 ± 0.57 | 0.65 ± 0.08 |

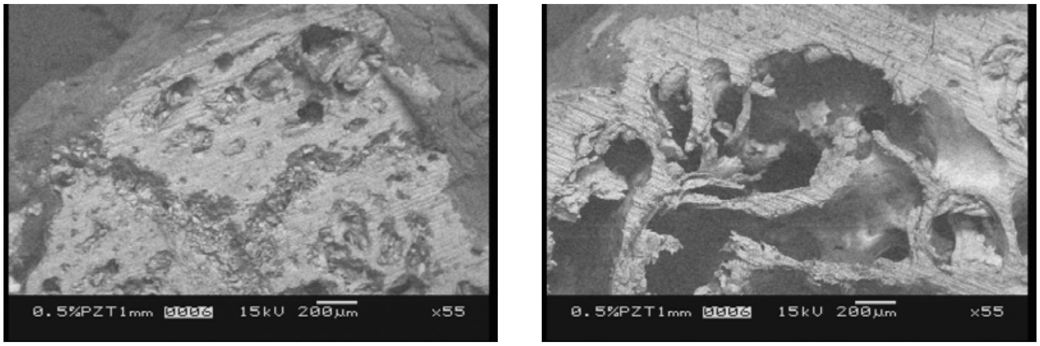
The morphological differences between the normal and osteoporotic bones were evident from the images obtained from micro-CT as shown in figure 3 while those obtained from scanning electron microscopy (SEM) are shown in figure 4. The normal bone showed a thicker trabecular structure with smaller marrow spaces and higher bone density whereas the osteoporotic bone had thinner trabecular structure with wider marrow space and lower bone mass density. The trabecular thickness and separation obtained from the three-dimensional model from micro-CT is shown in Table 2. The osteoporotic bones showed a trend of lower trabecular width (35%) and higher trabecular separation (85%). This is similar to that reported by Yang et al. [4].



normal

osteoporotic

Figure 3: Microtomography image of normal and osteoporotic bone



Normal Osteoporotic

Figure 4: SEM images of normal and osteoporotic bones

Table 2: Trabecular thickness and separation of section of rat femur

| | Rat | Thickness/ mm | Separation/ $mm^{-1}$ |
|---|---|---|---|
| **Normal bone** | **N1** | 0.1289 | 0.1659 |
| | **N2** | 0.1159 | 0.1727 |
| | **N3** | 0.1302 | 0.1696 |
| | **N4** | 0.1324 | 0.1569 |
| | **N5** | 0.1158 | 0.1579 |
| | **Mean ± S.D** | 0.1246 ± 0.00812 | 0.1646 ± 0.00701 |
| **Osteoporotic bone** | **O1** | 0.0823 | 0.3022 |
| | **O2** | 0.0870 | 0.2891 |
| | **O3** | 0.0713 | 0.2766 |
| | **O4** | 0.0928 | 0.3237 |
| | **O5** | 0.0694 | 0.3313 |
| | **Mean ± S.D** | 0.0806 ± 0.01006 | 0.3046 ± 0.02294 |

The results of the ANOVA test are shown in Table 3. The value of $F_{0.05}$ with 1 and 8 degrees of freedom is 5.32. Although the mean maximum load is lower for the osteoporotic bone than the normal bone, it wasn’t statistically significant. However it is significant that osteoporosis had an effect on the Young’s modulus, hardness, trabecular thickness and trabecular separation of the rat femur. More interestingly the value of F for the Young’s modulus and hardness are lower than that of the trabecular thickness and separation. Osteoporosis has been known to result in morphological changes whereas the sub-micron level mechanical properties were less explored. The results showed that both changes occurred in osteoporotic bone but the morphological changes are noted to be more significant.

Table 3: Value of $F_{0.05}$ of various properties measured

| Material and morphological properties | Value of $F_{0.05}$ |
|---|---|
| Maximum load | 2.55 |
| Young’s modulus | 45.88 |
| Hardness | 42.74 |
| Trabecular thickness | 58.10 |
| Trabecular separation | 170.18 |

The above study can benefit from the following improvements that can be made. Firstly, the normal and osteoporotic rats should be of the same age when sacrificed so as to exclude the effect of physiological changes on bone property. Secondly, the experiments which had been conducted over a period of several weeks should preferably be conducted immediately after sacrifice so as to minimize the effect of bone degradation.

## 4 CONCLUSIONS

Nanoindentation and microtomography are reliable techniques in assessing the quality of bone at the micron and nanometer scale. In addition, the findings of more obvious changes in morphology at the micron level might help in more accurate or earlier diagnosis of osteoporosis. It can also be used to study the efficacy of various treatment methods.

## 5 REFERENCES

1) Cowin, S., “Bone Mechanics,” CRC Press, pp 20-6 – pp 20-9, 2001

2) Oliver, W.C., Pharr, G.M., “An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments,” Journal of Materials Research, Vol. 7, pp 1564-1583, 1992.

3) Jamsa, T., Rho, J.Y., Fan, Z., Mackay, C. A., Marks Jr. S. C., Tuukkanen, J., “Mechanical properties in long bones of rat osteopetrotic mutations,” Journal of Biomechanics, Vol. 35, pp 161-165, 2002.

4) Yang, J., Pham, S.M., Crabbe, D.L., “Effects of oestrogen deficiency on rat mandibular and tibial microarchitecture,” Dentomaxillifacial Radiology, Vol. 32, pp 247-251, 2003.